Effect of Dehydration on the Formation of Levofloxacin Pseudopolymorphs

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Differential scanning calorimetry (DSC) curves of levofloxacin hemihydrate measured under various conditions showed different thermograms. These phenomena were attributed to be the dehydration. Dehydration caused a multiple-phase transition. Dehydration at a higher temperature (above 70 °C) gave a sharp endothermic peak on the DSC curve due to the melting of the γ form, and at a lower temperature (below 50 °C) gave a sharp endothermic peak due to the melting of the α form.

In contrast, the thermal behavior of levofloxacin monohydrate was not affected by dehydration. The difference in the thermal behavior between the hemihydrate and the monohydrate might be attributed to a difference in the interaction between levofloxacin and crystal water. Observations by thermomicroscopy, the changes in powder X-ray diffraction patterns during heating, and single X-ray analysis all supported the above interpretation.

Key words dehydration; thermal analysis; phase transition; polymorph; single X-ray analysis; thermomicroscopy

Levofloxacin hemihydrate ((−)-(*S*)-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7*H*-pyrido[1,2,3-de]-[1,4]benzoxazine-6-carboxylic acid hemihydrate) (Chart 1) is an optical *S*-isomer of ofloxacin which is widely used in the world as an antibacterial agent. Levofloxacin was generally twice as potent as ofloxacin against a variety of gram-positive and gram-negative pathogens.[1)]

Levofloxacin exists in at least two modifications, the hemihydrate and the monohydrate. It is well known that different polymorphs and hydrates of drugs exhibit different physicochemical properties such as phase stability and solubility.[2)] Levofloxacin hemihydrate is superior to levofloxacin monohydrate as a drug bulk substance in terms of phase stability.[3)]

However, differential scanning calorimetry (DSC) curves of levofloxacin hemihydrate measured under various conditions showed 1—3 endothermic peaks corresponding to the melting point, while those of levofloxacin monohydrate showed only one endothermic peak. Investigation of the thermal properties is very important since differential scanning calorimetry is often used as a tool for routine quality control. The objects of the present work were to examine the thermal properties of levofloxacin hemihydrate and monohydrate by means of DSC, thermogravimetry-differential thermal analysis (TG-DTA) and video DSC hot stage microscopy (thermomicroscopy). Powder X-ray diffractometry and single crystal X-ray diffraction analysis were also performed.

Experimental

Materials Levofloxacin hemihydrate was of pharmaceutical grade and was synthesized in our laboratory.

Preparation of Levofloxacin Monohydrate A slurry of 10 g of levofloxacin hemihydrate and 100 ml of water was stirred at room temperature for 24 h. The crystals were collected by filtration and washed with 20 ml of water. The crystals were dried under reduced pressure at 80 °C for 4 h. After being dried, the crystals were kept at about 60% relative humidity overnight. 8.3 grams of levofloxacin monohydrate was recovered, representing a yield of 83%.

Measurement of Water Content The water content of the samples was determined by the Karl Fischer method (type MK-II, Kyoto Denshi Kogyo).

DSC DSC thermograms of the sample were recorded on a DuPont 910 differential scanning calorimeter connected to a DuPont 9900 computer. The system was able to calculate the extrapolated onset temperature, peak temperature and enthalpy values for each thermal reading. The temperature axis was calibrated with pure indium, with a melting point of 156.60 °C. The lids of sample pans were not used in order to allow water to escape during dehydration under a nitrogen atmosphere. An empty pan was used as a reference. Heating rates (1—20 °C/min) and nitrogen gas flow rates (30—100 ml/min) were varied according to the purpose of the experiment.

Thermogravimetric Analysis (TGA) DTA and TG were performed using a type SSC/5200 TG/DTA 220 (Seiko Instruments). The operating conditions in an open-pan system were as follows: sample weight, 10 mg; heating rate, 10 °C/min; and N_2 gas flow rate, 100 ml/min.

Powder X-Ray Diffraction Patterns The powder X-ray diffraction patterns of the samples were obtained using a Mac Science Model MXP-3V diffractometer. The operating conditions were as follows: target, Cu; filter, Ni; Voltage 35 kV; Current, 20 mA; receiving slit, 0.15 mm; time constant, 1 s; and scanning speed, 1° 2θ/min. Powder X-ray diffractometry at a high temperature of 108 °C was carried out using the same diffractometer equipped with a Mac Science temperature attachment and controller. After measuring the diffraction at room temperature, the temperature was raised to 108 °C at a heating rate of 10 °C/min, and then the temperature was raised to 165, 184, 203, 213 and 222 °C. Each appointed temperature was maintained for 25 min.

Single-Crystal X-Ray Analysis Unit cell determination and atomic coordinates were determined using a Rigaku AFC5R diffractometer with graphite-monochromated Cu$K\alpha$ radiation and a 12KW rotating anode generator. A colorless prism crystal of the hemihydrate having approximate dimensions of $0.300 \times 0.300 \times 0.300$ mm and a yellow plate crystal of the monohydrate having approximate dimensions of $0.500 \times 0.200 \times 0.050$ mm were mounted on a glass fiber. Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections. The intensities were corrected for Lorentz and polarization factors, but not for absorption or extinction effects. Structure refinement was carried out using a DIRDIF program utilizing full-matrix least-squares techniques. Final models included all nonhydrogen atoms which were thermally anisotropic.

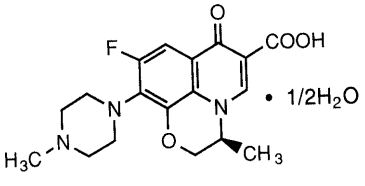

Chart 1. The Structure of Levofloxacin Hemihydrate

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Results and Discussion

Identification of Pseudopolymorphs The results of elemental analysis and water content for the pseudopolymorphs are shown in Table 1. The observed values in elemental analysis and water content coincided with the theoretical values, as shown in Table 1.

The powder X-ray diffraction patterns of the hemihydrate and the monohydrate are shown in Figs. 2 and 3. The powder X-ray diffraction patterns of the pseudopolymorphs showed different patterns. Characteristic diffraction peaks of the pseudopolymorphs were observed at $2\theta = 6.5$, 12.9° and at 7.8, 11.3° for the hemihydrate and the monohydrate, respectively.

Thermal Behavior Typical TG-DTA curves of the hemihydrate and monohydrate are shown in Fig. 1. The DTA curve of the hemihydrate showed an endothermic peak at 74.2 °C, with 2.4% of the weight loss due to the dehydration of 1/2 mol of water per mol of the drug (Calcd 2.43%), and three endothermic peaks at 227.1, 231.1 and 234.0 °C. The weight loss due to the degradation of levofloxacin after melting was observed on the TG curve. The DTA curve of the monohydrate showed an endothermic peak at 65.2 °C with 4.5% of the weight loss due to the dehydration of 1 mol of water per mol of the drug (Calcd 4.74%), and the melting peak was at 234.3 °C.

Thermomicroscopy At a heating rate of 10 °C/min, we observed that the hemihydrate began to melt at about 225 °C and the majority of crystals melted at about 228 °C. However, before it completely liquefied, a new solid phase began to partially crystallize, and then the new solid phase was seen to melt at approximately 234 °C. The melting pattern of the hemihydrate was complicated, whereas the monohydrate melted at *ca.* 234 °C.

When the hemihydrate melted completely at 234 °C and was then quenched rapidly to room temperature, we observed the partial crystallization of small particles from the vitreous phase. Crystallization of the whole vitreous phase occurred gradually at room temperature. To determine the level of impurities, HPLC was conducted using an ODS column and a 0.05 M phosphate buffer (pH 2.4)/methanol (3 : 1, v/v) solution as the elution solvent. Analysis by HPLC showed that this material was 99.6% pure compared with untreated levofloxacin and no degradation products were detected. Powder X-ray diffraction analysis revealed that this material was a mixture of the hemihydrate and the monohydrate.

These observations indicated that the hemihydrate underwent multiple-phase changes during thermomicroscopic investigation.

Powder X-Ray Diffraction Analysis Changes in the powder X-ray diffraction patterns of the hemihydrate and the monohydrate by heating are shown in Figs. 2 and 3, respectively. As seen in Figs. 2 and 3, the power X-ray diffraction patterns of the hemihydrate and the monohydrate varied with temperature. The samples after heating above 108 °C were confirmed to be anhydrates by the determination of their water content using the Karl Fisher method. Above 213 °C, peak intensities decreased as the result of degradation of the anhydrates. The anhydrates formed from the hemihydrate and the monohydrate after heating at 108 to 203 °C exhibited characteristic patterns and could be distinguished from one another. Therefore, these anhydrates were named the γ form and α form, respectively. These γ and α forms adsorbed water vapor rapidly under ordinary relative humidity conditions and transformed into the hemihydrate and the monohydrate, respectively.

From the results of thermal analysis, it can be concluded that the γ form melts at 226 °C and the α form melts at 234 °C. The results of thermal analysis suggested the existence of another crystal form with a melting point of 230 °C, and this crystal form was named the β form.

Single Crystal X-Ray Analysis Crystal data for the hemihydrate and the monohydrate are listed in Table 2. The molecular packing diagrams of the hemihydrate and the monohydrate are shown in Figs. 4 and 5, respectively. The crystal structures of the hemihydrate and the monohydrate were nonisomorphous. In the crystal structure of the hemihydrate, the water molecules appear to have a weaker interaction with a lone pairing of methylammonium nitrogen. On the other hand, the oxygen of carboxylic acid accepts a hydrogen bond from a water molecule for the crystal structure of the monohydrate. These differences might cause the formation of polymorphs of the anhydrates from the hemihydrate and the monohydrate by heating.

Effect of the Atmospheric Conditions on the DSC Curve DSC curves of the hemihydrate are shown in Fig. 6 under the nitrogen gas flow at flow rates of 30 and 100 ml/min.

DSC curve at a gas flow rate of 30 ml/min indicated

Table 1. Elemental Analysis and Water Content of the Pseudopolymorphs

Pseudopolymorph		Elemental analysis (%)				Water content (%)
		C	H	F	N	
Hemihydrate	Calcd	58.37	5.71	5.13	11.35	2.43
	Found	58.32	5.43	5.27	11.37	2.40
Monohydrate	Calcd	56.99	5.84	5.01	11.08	4.74
	Found	57.02	5.95	4.93	11.01	4.68

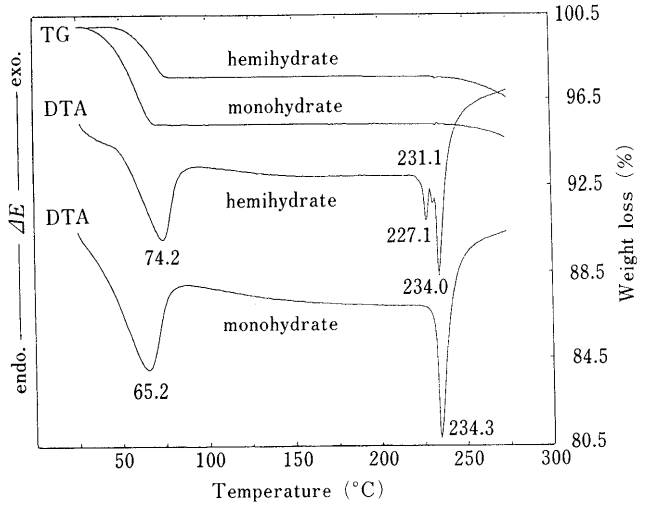

Fig. 1. TG and DTA Curves for the Hemihydrate and the Monohydrate

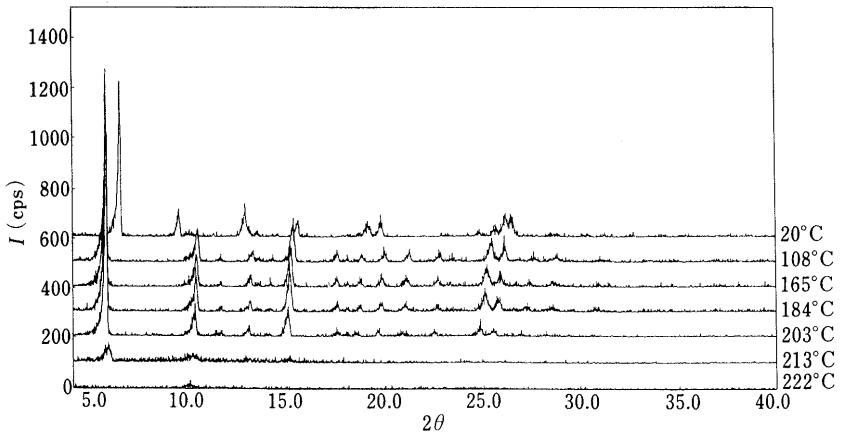

Fig. 2. Change of Powder X-Ray Diffraction Patterns of the Hemihydrate by Heating

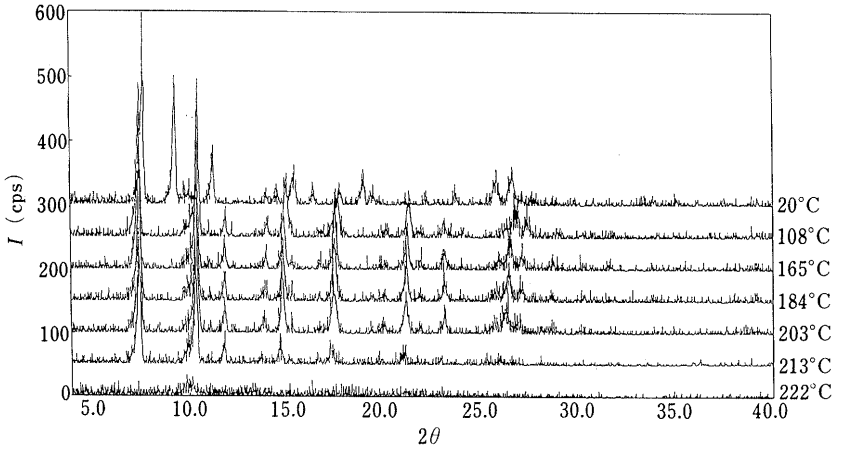

Fig. 3. Change of Powder X-Ray Diffraction Patterns of the Monohydrate by Heating

Table 2. Crystal Data of Levofloxacin Hydrates

	Hemihydrate	Monohydrate
Space group	C_2	$F2_1$
System	Monoclinic	Monoclinic
Unit cell dimensions		
a (Å)	29.092	6.854
b (Å)	6.879	13.911
c (Å)	18.838	18.544
β (°)	114.064	95.47
Unit cell volume, V (Å^3)	3442.3	1760.0
Unit per cell, Z	8	4

two endothermic peaks, at 70.7 and 227.3 °C, that were due to the dehydration and melting of the γ form, respectively. Furthermore, the DSC curve showed an exothermic peak at 225.7 °C, followed by the melting peak. This exothermic peak was considered to be due to the crystallization of an amorphous form which resulted partially from the dehydration.

In contrast, DSC curve measured at a gas flow rate of 100 ml/min indicated an endothermic peak at 66.0 °C due to the dehydration, an endothermic peak at 227.8 °C due to the melting of the γ form, an endothermic peak at 231.6 °C due to the melting of the β form and an endothermic peak at 234.1 °C due to the melting of the α form. The dehydration temperature measured at the gas flow rate of 100 ml/min was a little bit lower than that measured at the gas flow rate of 30 ml/min. The reason the dehydration occurred at the lower temperature might be due to the quick removal of the water vapor generated by the dehydration caused by rapid gas flow. Moreover,

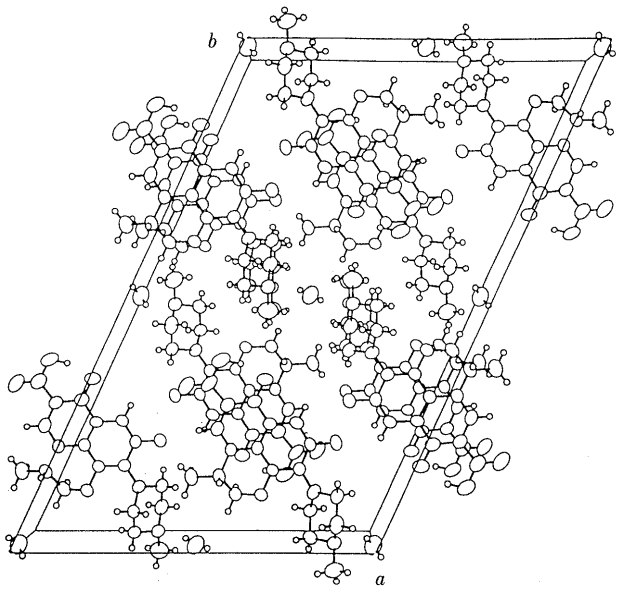

Fig. 4. Stereoscopic Drawing of the Molecular Packing Viewed along the c-Axis for the Hemihydrate

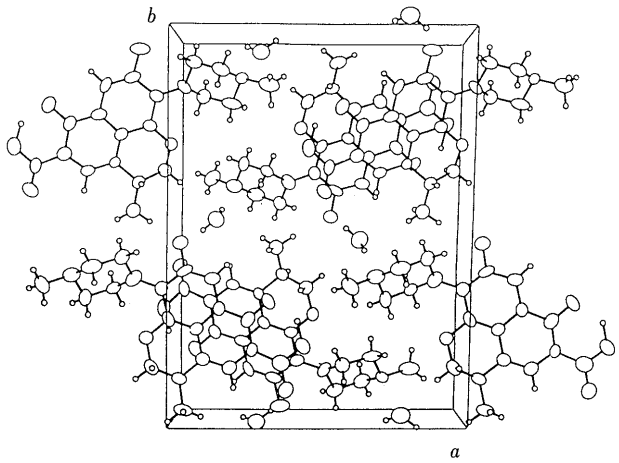

Fig. 5. Stereoscopic Drawing of the Molecular Packing Viewed along the *c*-Axis for the Monohydrate

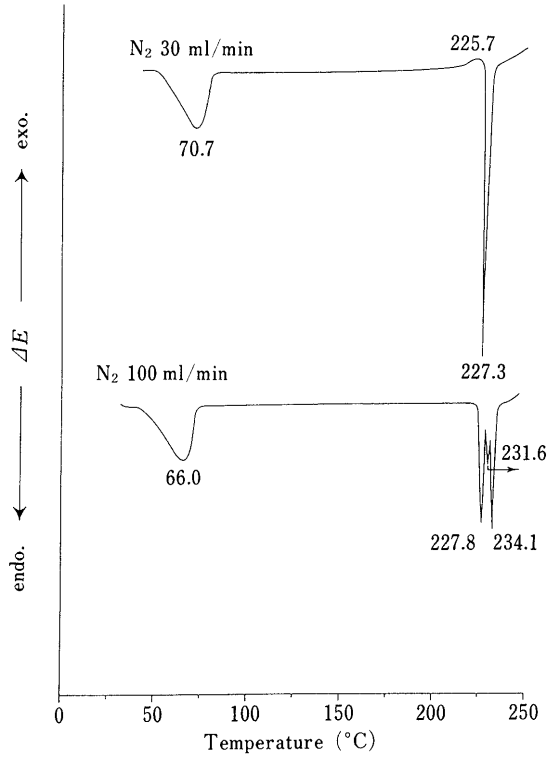

Fig. 6. Effect of N_2 Gas Flow Rate on DSC Curves of Levofloxacin Hemihydrate

Heating rate, 10 °C/min.

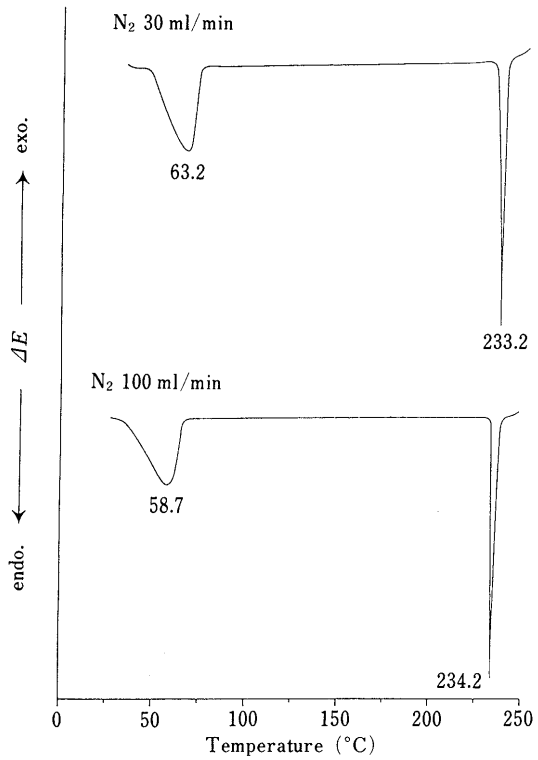

Fig. 7. Effect of N_2 Gas Flow Rate on DSC Curves of Levofloxacin Monohydrate

Heating rate, 10 °C/min.

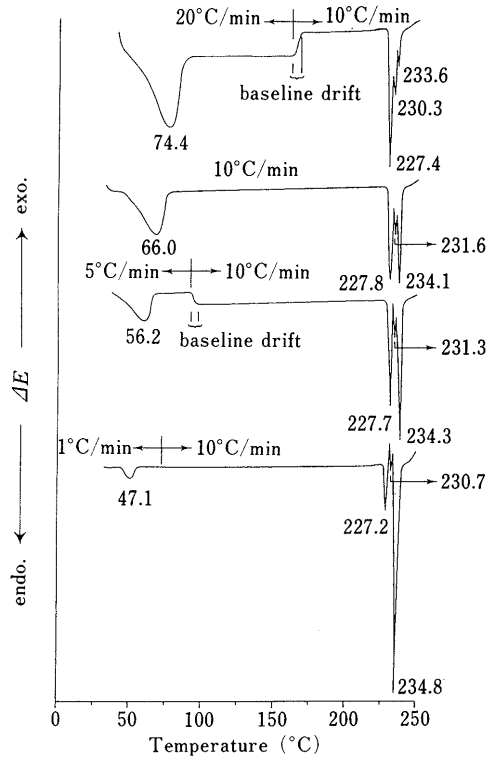

Fig. 8. Effect of Heating Rate on DSC Curves of Levofloxacin Hemihydrate

N_2 gas flow rate, 100 ml/min.

no exothermic peak was observed on the DSC curve measured at the gas flow rate of 100 ml/min, and it was considered that little formation of the amorphous form occurred under a sufficient nitrogen purge.

On the other hand, the endotherm for the melting of the monohydrate was not affected by the nitrogen gas flow rate (Fig. 7).

It is considered that the effect of nitrogen gas was to purge the water vapor generated from the hemihydrate, preventing the formation of the amorphous phase and promoting the formation of seeds of polymorphs. We assumed that these phenomena were attributed to a change in the dehydration mechanism as the water vapor partial pressure increased. Thus, under an atmosphere of sufficient nitrogen purge, the dehydration occurred rapidly and then resulted in anhydrate crystals with little lattice defect and/or lattice disorder. However, under an atmosphere of insufficient nitrogen purge, the dehydration depressed by

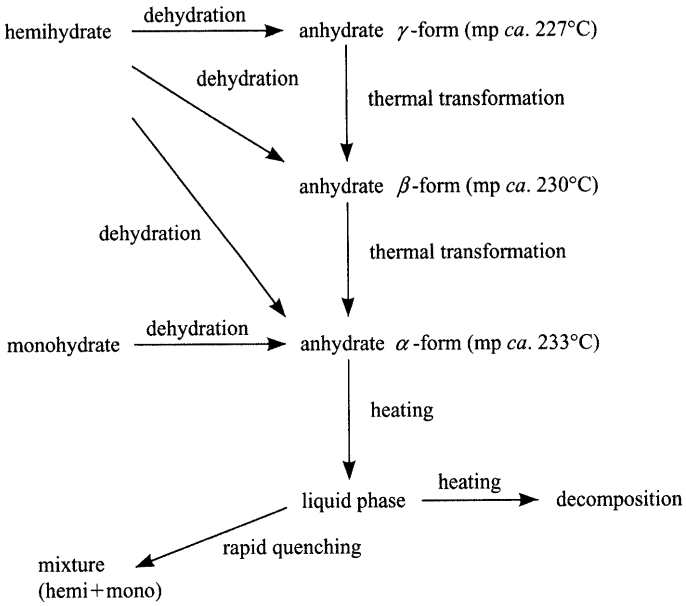

Chart 2. Possible Interconversion of Levofloxacin Hydrates and Polymorphs

the water vapor partial pressure caused a burst of crystal surface and then resulted in anhydrate crystals with significant lattice defects and/or lattice disorder.

The difference in thermal behavior between the hemihydrate and the monohydrate might be attributed to the difference of interaction between levofloxacin and crystal water.

Effect of the Heating Rate on the DSC Curve The effect of different heating rates on the DSC thermograms of the hemihydrate are illustrated in Fig. 8.

When the hemihydrate was heated from room temperature to about 160 °C at a heating rate of 20 °C/min and then at 10 °C/min, the endotherm due to the dehydration was observed at a higher temperature (74.4 °C) than that (66.6 °C) measured at 10 °C/min. When the dehydration occurred at a higher temperature, the endotherm due to the melting of the γ form was detected sharply on the DSC curve. At 5 °C/min, from room temperature to about 90 °C, and then 10 °C/min, the peak temperature of dehydration was lower (56.2 °C), and three endotherms due to the melting of three pseudopolymorphs were observed. At 1 °C/min from room temperature to about 70 °C, and then at 10 °C/min, the dehydration occurred at 47.1 °C. When the dehydration occurred at a lower temperature, the endothermic peak at 234.8 °C due to the melting of the α form became sharper. These results showed that the dehydration at a lower temperature resulted in partial lattice collapse and then recrystallization of the crystal lattice to give the α form. However, it was apparent that the dehydration did not always lead to lattice collapse and recrystallization of the α or β form.

From the above findings, the possible mechanism for the formation of the pseudopolymorphs is summarized in Chart 2.

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