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Studies on mechanism of thermal crystal transformation of sitafloxacin hydrates through melting and recrystallization, yielding different anhydrates depending on initial crystalline forms

Tetsuya Suzuki[a,b,*], Tetsuya Araki[a], Hiroaki Kitaoka[c], Katsuhide Terada[b]

[a] Analytical & Quality Evaluation Research Laboratories, Pharmaceutical Technology Division, Daiichi Sankyo Co., Ltd., 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan
[b] Department of Pharmaceutics, School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan
[c] Analytical Group II, Research Department II, Daiichi Sankyo RD Associe Co., Ltd., 3-10-2 Kitashinagawa, Shinagawa-ku, Tokyo 140-0001, Japan

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ABSTRACT

The polymorphic and pseudopolymorphic forms of sitafloxacin, a novel fluoroquinolone antibiotic, were characterized by infrared spectroscopy, X-ray diffractometry, and thermal analysis. Hydrates of sitafloxacin underwent thermal transformation during the course of heating to 300 °C. Monohydrate melted at 130 °C and crystallized at 147 °C to yield α-form (anhydrate) while sesquihydrate melted at 127 °C and crystallized at 146 °C to yield β-form (anhydrate). The crystal structural analysis revealed that monohydrate and sesquihydrate had opposite torsion at quinolone ring and the conformation of quinolone ring tended to be retained during hydrates to anhydrates crystal conversion. The infrared spectroscopy showed that hydrates and anhydrate α-from exists in zwitterion while β-from is consist of neutral molecule. Detail investigation of thermal behavior of hydrates suggested that water vapor also affected anhydrous crystal forms obtained by heating hydrates, though promoting ionization at carboxyl group and amine group.

1. Introduction

Fluoroquinolone antibacterial agents have been widely used as therapeutic agents for general bacterial infections. Sitafloxacin (STFX): (7-[(7*S*)-7-amino-5-azaspiro[2.4]heptan-5-yl]-8-chloro-6-fluoro-1-[(1*R*,2*S*)-2-fluoro-1-cyclopropyl]-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid) (Fig. 1) is a new fluoroquinolone which is recently put on the market with a trade name of Gracevit® (Daiichi-Sankyo Co., Ltd.). Its antibacterial activity is significantly better than those of other fluoroquinolones (Sato et al., 1992; Touyama et al., 2006; Okuda et al., 2007).

Polymorphism of an active pharmaceutical ingredient (API) may have an influence on various natures, e.g., solubility, bioavailability and stability (Haleblian and McCrone, 1969; Jasti et al., 1995; Rocco et al., 1995; Ito et al., 1997; Kojima et al., 2007a,b). Therefore, investigations of polymorphs and pseudopolymorphs, e.g., exploring and identification of polymorphs and pseudopolymorphs, comparisons of physicochemical properties of such forms and clarifying transformation between crystalline forms, are an important part of preformulation study. Thermal analysis has been widely used to identify crystal forms of APIs and in the course of thermal analysis, crystal transformation is often observed as well as melting and decomposition.

In the case of monotropic polymorphism, a crystal yielded by heating is a stable form not only at higher temperature but also at room temperature.

It is reported that carbamazepine form II (anhydrate) converted into form III (anhydrate) at around 135 °C, exhibiting a broad exothermic peak on a differential thermal analysis (DTA) curve. DTA curves of other crystal forms of carbamazepine including form I (anhydrate), form IV (dihydrate) and form V (acetone solvate) also suggested transformation into form III. Form III did not show crystal conversion stored below 91% relative humidity (RH) for 28 days (Kaneniwa et al., 1984). This implies that thermal analysis is useful for searching a stable crystal form. When a hydrate is selected as a drug substance in clinical use, it is essential to investigate thermal dehydration and subsequent crystal conversion, because a drug substance could be exposed to dry air at high temperature during conventional formulation process, such as drying step of granules. Thermal analysis is often also used as a routine tool for quality control. Therefore, comprehension of thermal property is very important from early stage of development through the marketed stage.

In this study we prepared and characterized two anhydrates (α-from and β-from) and two hydrates (hemihydrate and monohydrate) in addition to sesquihydrate which is used in the marketed

* Corresponding author at: Analytical & Quality Evaluation Research Laboratories, Pharmaceutical Technology Division, Daiichi Sankyo Co., Ltd., 1-16-13 Kitakasai, Edogawa-ku, Tokyo 134-8630, Japan. Tel.: +81 3 3680 0151; fax: +81 3 5696 8035.
E-mail address: tetsuya.suzuki.fb@daiichisankyo.co.jp (T. Suzuki).

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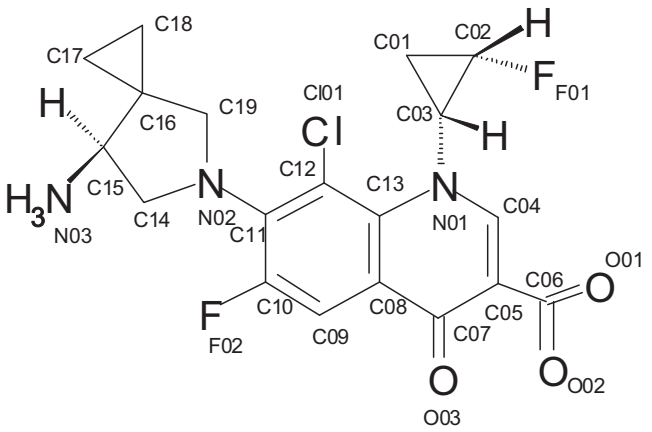

Fig. 1. Chemical structure and numbering of molecules and atoms in sitafloxacin.

drug product. Especially, thermal behaviors of hydrates were examined extensively. Crystal structural analysis threw new light on dehydration and crystal transformation during heating.

2. Experimental

2.1. Materials

STFX sesquihydrate was obtained from Daiichi-Sankyo Co., Ltd. (Tokyo, Japan). All solvents were purchased from Wako Pure Chemical Industries (Osaka, Japan) or Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2. Sample preparations

Small amount of STFX sesquihydrate for single crystal X-ray analysis was prepared by recrystallizing from an aqueous solution. Anhydrate α-from was obtained by recrystallizing from ethanol. About 100 mg of sesquihydrate was added to 100 mL of boiling ethanol. After stirring vigorously, residual solid was removed by filtration and anhydrate α-from was precipitated by cooling filtrate. Anhydrate β-from was prepared by slurring about 1 g of sesquihydrate in 50 mL of toluene at the boiling temperature for about 1 h. Hemihydrate was yielded by slurring about 0.5 g of sesquihydrate in 100 mL of 2-propanol/water (99:1, v/v) at the boiling temperature (reflux condition). All crystal forms described above were filtered and dried in a nitrogen atmosphere. Monohydrate was prepared as follows: excess amount of sesquihydrate was added to 100 mL of methanol at about 70 °C and stirred for a few minutes. After residual solid was removed by filtration, the monomethanolate was recrystallized from the filtrate. The monomethanolate was filtered and converted to monohydrate by storing at an ambient condition (approximately 25 °C/30–70% RH) for about 3 days.

2.3. Infrared spectra

FT-IR spectra were recorded using potassium bromide disk method at 2 cm^{-1} resolution from 400 to 4000 cm^{-1} on an FT720 (Horiba, Kyoto, Japan).

2.4. X-ray powder diffraction (XRPD) analysis

X-ray powder diffraction patterns were measured using an X'pert Pro powder X-ray diffractometer (PANalytycal, Almelo, Netherland) with Cu Kα radiation generated at 40 kV and 55 mA at room temperature. Data were collected between 5° and 40° 2θ with a step size of 0.015° and a scanning speed of 1° min^{-1}.

2.5. Single-crystal X-ray structure analysis

All form except hemihydrate were analyzed in this study, even β-form and sesquihydrate were published previously (Yamazaki and Suzuki, 1998; Suzuki et al., 2000). Single crystal X-ray diffraction data were collected on an AFC 7R diffractometer (Rigaku, Tokyo, Japan) or RAXIS-RAPID (Rigaku, Tokyo, Japan) with Cu Kα radiation at room temperature except for sesquihydrate (−150 °C).

The crystal structure was solved and refined using Crystal Structure software 3.6.0 (Rigaku, Tokyo, Japan).

2.6. Ab initio powder diffraction analysis

Anhydrate α-from sample was gently ground, by using an agate mortar and pestle, then mounted in a 0.3 mm borosilicate capillary tube to reduce preferred orientation effects.

The XRPD pattern was collected at BL02B in the Spring-8, the large synchrotron radiation facility of the Japan Synchrotron Radiation Research Institute (JASRI) located in Hyogo, Japan. The diffraction data was collected at the range of 1–70° (2θ) at a step size of 0.01°. The wavelength of radiation was set to 0.8 Å and calibrated by using cerium oxide (ceria: cubic, a = 5.4112 Å).

The structure solution was carried out using the program Reflex Plus 4.3 (Accelrys, San Diego, CA, USA). The diffraction pattern was indexed using X-Cell to obtain lattice parameters that were subsequently optimized by Pawley refinement.

Simulated annealing (SA) moves were performed with a slight modification because default run of SA does not optimize the torsion of quinolone ring and conformation of 5-menbered ring at 7-position. The bonds of C04-C05, C10-C11 of quinolone ring and a bond of C14-C15 of 5-menbered ring at 7-position were neglected during SA.

A final refinement was performed by Rietvelt method.

2.7. Thermal analysis

Thermogravimetry-differential thermal analysis (TG/DTA) was performed with TG/DTA 6200 system (SII NanoTechnology Inc, Tokyo, Japan). The sample (*ca.* 4 mg) was weighed into an open aluminum pan and then heated at rate of 10 °C/min under 100 mL/min of nitrogen flow.

2.8. Crystal transformation by heating

To confirm crystal form that were generated by heating of hydrates, about 20 mg of each hydrates was heated in an open aluminum pan and a sealed aluminum pan by DSC 6200 system (SII NanoTechnology Inc, Tokyo, Japan) at the heating rate of 10 °C/min under 100 mL/min of nitrogen flow.

After heating just above melting point, each hydrate was immediately cooled to room temperature and mounted on reflection free sample plate and XRPD pattern was measured with Rint 2000 series (TTR-III) diffractometer (Rigaku, Tokyo, Japan) with Cu Kα radiation generated at 50 kV and 300 mA.

3. Results and discussion

3.1. Infrared spectra

Infrared (IR) spectra obtained from five forms of STFX are presented in Fig. 2. Differences among these polymorphic forms can be seen in whole spectral region, especially in the range of 1400–1750 cm^{-1} which was assigned to be carbonyl and carboxylic acid group.

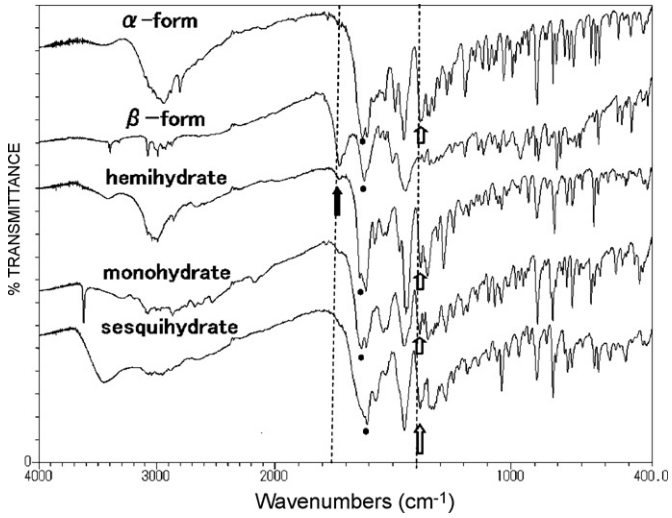

Fig. 2. Infrared spectra of polymorphs and pseudopolymorphs of sitafloxacin. Closed arrow: C=O of carboxylic acid, open arrows: C=O of carboxylate, closed circles: C=O of pyridone.

Anhydrate β-form showed two absorption bands for C=O at 1750 cm^{-1} and 1650 cm^{-1}.

On the other hand, other four forms showed absorption for C=O at around 1650 cm^{-1} and 1400 cm^{-1}.

Assignments of IR spectra for norfloxacin (Hu et al., 2002) and ciprofloxacin (Turel et al., 1997) were already reported. The absorption bands about 1730 cm^{-1} (norfloxacin) and 1707 cm^{-1} (ciprofloxacin) were assigned to C=O of carboxylic acid. It is also reported that the carboxylate (COO^-) did not exhibit absorption band around 1700 cm^{-1}, but instead showed two absorption band in the ranges 1610–1550 cm^{-1} and 1400–1280 cm^{-1}.

According to these findings, the absorption band at 1750 cm^{-1} was assigned to carboxylic acid and those at 1400 cm^{-1} were assigned to be carboxylates. Absorption bands at around 1600 cm^{-1} were assigned to mainly C=O of pyridone, though several absorptions were overlapped in this region.

IR spectra of STFX clearly showed that carboxyl group of STFX was protonated in β-form and dissociated in other crystal forms. And it was considered that STFX exist in neutral molecule in β-form and exists in zwitterion in other crystal forms, though protonation of amino group was not clear from IR spectra.

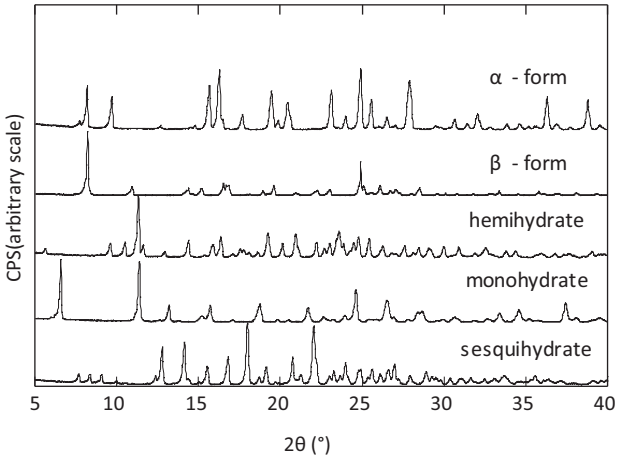

Fig. 3. X-ray powder diffraction (XRPD) patterns of polymorphs and pseudopolymorphs of sitafloxacin.

3.2. XRPD analysis

XRPD patterns for five forms are shown in Fig. 3. Significant differences were seen with respect to both of diffraction angle and intensity. Characteristic diffraction lines were observed at $2\theta = 8.2^\circ$ and 9.7° for α-form, $2\theta = 8.2^\circ$ and 10.9° for β-form, 5.8°, 11.3° for hemihydrate, 6.6° and 11.4° for monohydrate and 7.7°, 8.4° and 9.1° for sesquihydrate. These observations showed that these samples had different crystal structure and were readily distinguished by XRPD.

3.3. Crystal structure

STFX molecules in several crystal forms are shown in Fig. 4. Crystal parameters and experimental data are shown in Table 1. Crystal structural analysis for sesquihydrate (Suzuki et al., 2000) and β-form (Yamazaki and Suzuki, 1998) gave the same results as previously reported. The quinolone ring of STFX did not take planner conformation. The distortion of quinolone ring would arise from steric hindrance between chorine atom at the 8th position and cyclopropyl moiety at the 1st position. STFX β-form and sesquihydrate had similar torsion at quinolone ring, while STFX in monohydrate had opposite torsion to that of β-form and sesquihydrate. This means quinolone ring of STFX can take at least two conformations, namely, β-form type and monohydrate type. The crystal structure of α-form was determined from XRPD data

Table 1
Crystal parameters and experimental data for X-ray structural analysis.

	α-form	β-form	Monohydrate	Sesquihydrate
Empirical formula	$C_{19}H_{18}ClF_2N_3O_3$	$C_{19}H_{18}ClF_2N_3O_3$	$C_{19}H_{20}ClF_2N_3O_4$	$C_{19}H_{21}ClF_2N_3O_{4.5}$
Method	Powder	Single crystal	Single crystal	Single crystal
Sample mount	Packed in 0.3 mm diameter of borosilicate capillary	Mounted on glass fiber	Mounted on glass fiber	Mounted on glass fiber
Wavelength	0.802463 Å (synchrotron radiation)	1.54178 Å (Cu Kα X-rays)	1.54178 Å (Cu Kα X-rays)	1.54178 Å (Cu Kα X-rays)
Crystal system	Monoclinic	Orthorhombic	Monoclinic	Orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_1$	$P2_12_12_1$
Temperature	20 °C (room temp.)	23 °C (room temp.)	23 °C (room temp.)	−150 °C
a (Å)	11.710[a]	12.451(5)	8.374(3)	13.599(2)
b (Å)	7.140[a]	21.205(6)	8.149(4)	13.610(3)
c (Å)	11.044[a]	6.897(4)	13.682(2)	20.816(3)
α (°)	90	90	90	90
β (°)	105.12[a]	90	102.33(2)	90
γ (°)	90	90	90	90
V (Å^3)	891.5[a]	1820(1)	912.1(5)	3852(1)
Z	2	4	2	8
D_{calcd} (g/cm^3)	1.527	1.495	1.558	1.506
R or R_P	2.8%[b]	3.8%	6.3%	7.7%

[a] Standard deviation of lattice parameter was not estimated by XRPD analysis.
[b] R_P.

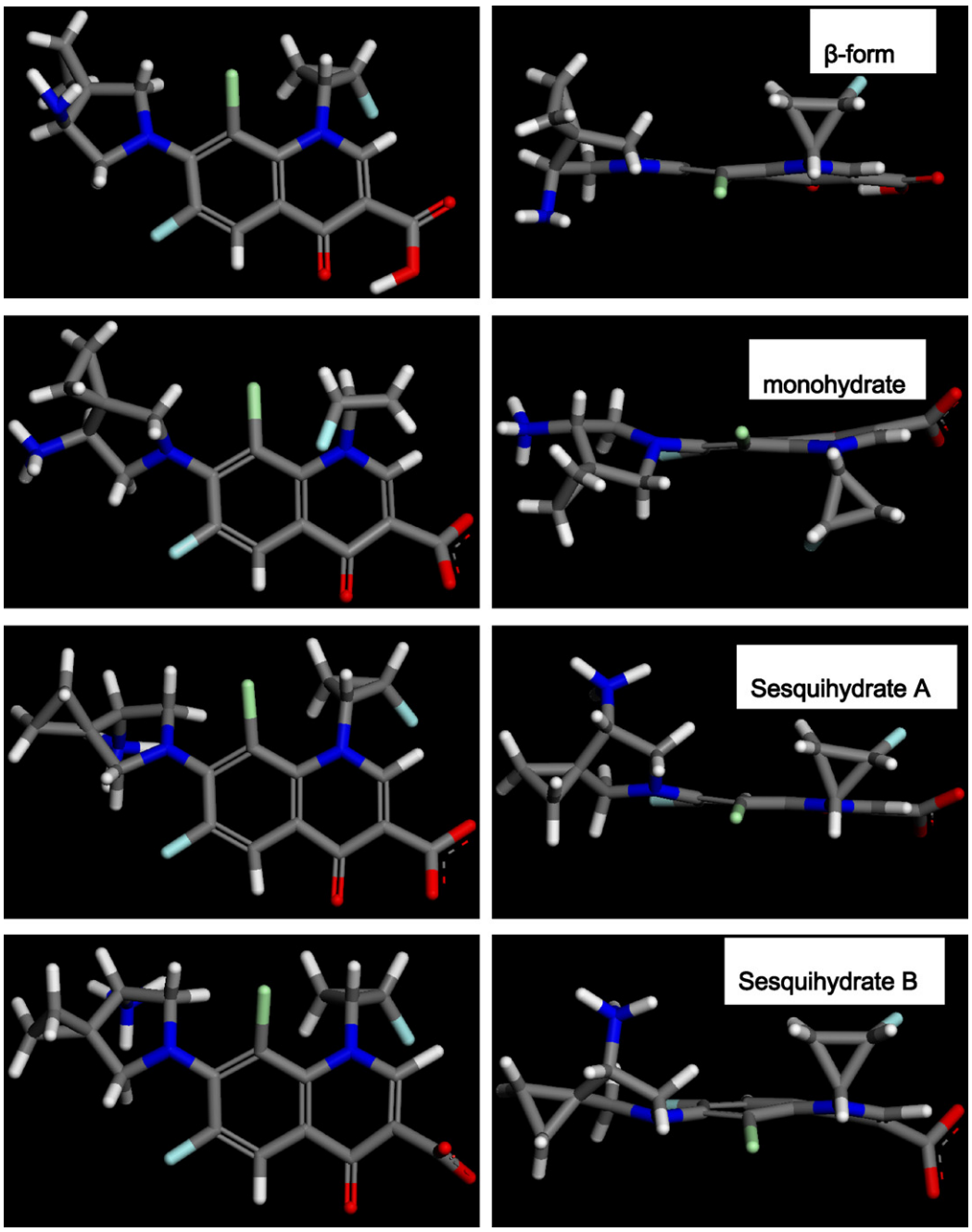

Fig. 4. Sitafloxacin molecule in β-from, monohydrate and sesquihydrate. Sesquihydrate contains two independent molecules in an asymmetric unit.

because single crystal of α-form has not been prepared despite of considerable effort. STFX molecule in α-form has similar structure with monohydrate. Crystal structure of α-form is shown in Fig. 5. The final Rietveld refinement plot is shown in Fig. 6. Final Rwp, and R_p values are 3.73% and 2.75%, respectively. The crystal structure of hemihydrate has not been determined because suitable single crystals for X-ray structure analysis have not been obtained. Crystal structural analysis from XRPD pattern is also quite difficult because of complexity of this crystal: lattice parameters suggested that four STFX molecules and two water molecule were contained in an asymmetric unit.

3.4. Thermal analysis

TG/DTA curves of five forms are shown in Fig. 7. The DTA curves of α-form and β-form showed endothermic peaks due to melting at approximately 240 °C and 220 °C, respectively. The TG curves of these samples showed no mass losses from room temperature to around melting point, suggesting no solvent were involved in α-form and β-form. Exothermic peaks and steep mass loss just above melting point indicated that STFX decomposed immediately after melting.

The DTA curve of hemihydrate showed two broad endothermic peaks at approximately 170 °C and 175 °C with 2.2% of the mass loss due to the dehydration (theoretical: 2.15%) and the crystal transformation to anhydrous crystal form. The hemihydrate was considered to be converted in to β-form, because the hemihydrate showed endothermic peak at around 220 °C where β-form melted and decomposed.

This interpretation was supported by XRPD patterns of STFX converted from hemihydrate by heating up to 190 °C (see below and Fig. 8).

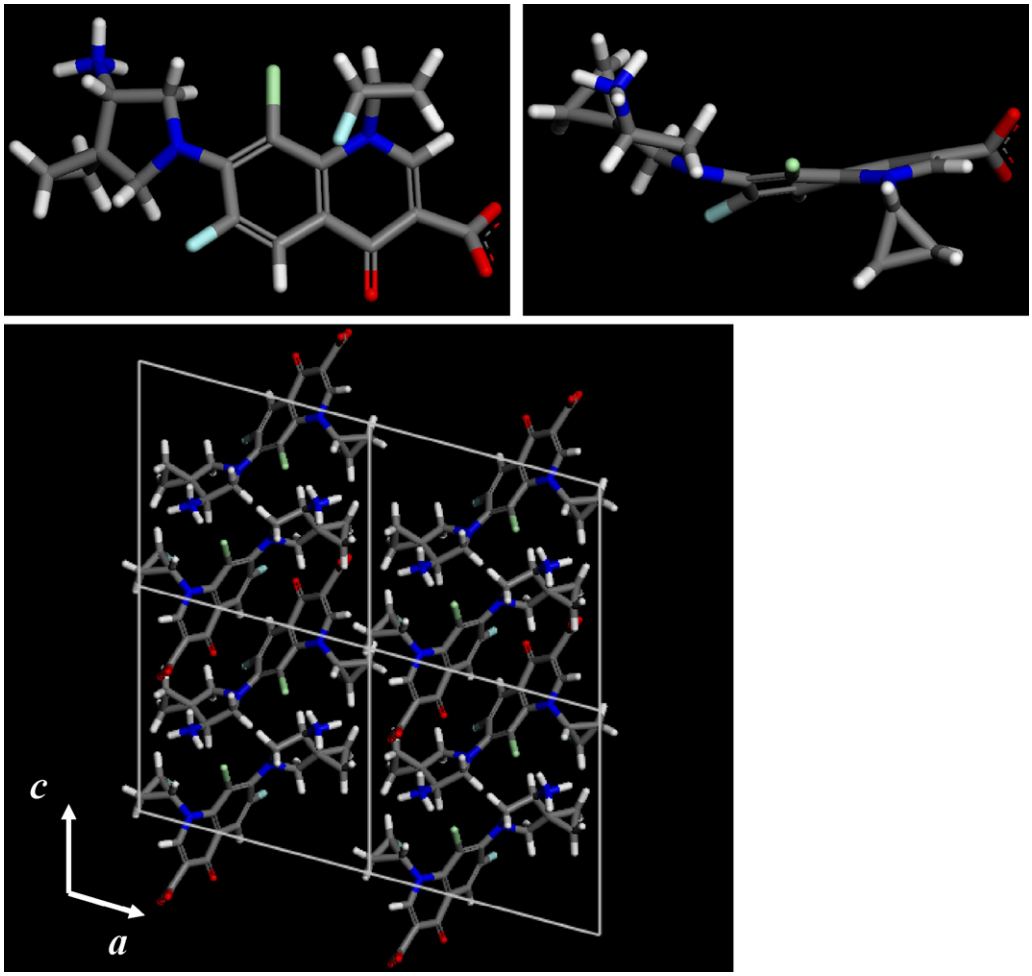

Fig. 5. Sitafloxacin molecule in α-from and crystal structure of α-from.

The DTA curve of monohydrate showed endothermic peaks at 100 °C, 130 °C and 240 °C and an exothermic peak at 147 °C. The TG curve of monohydrate showed 4.1% of the mass loss due to the dehydration (theoretical: 4.21%).

These observations indicated that the monohydrate was dehydrated below around 100 °C and converted to α-form by melting and recrystallization in the range of 130–150 °C.

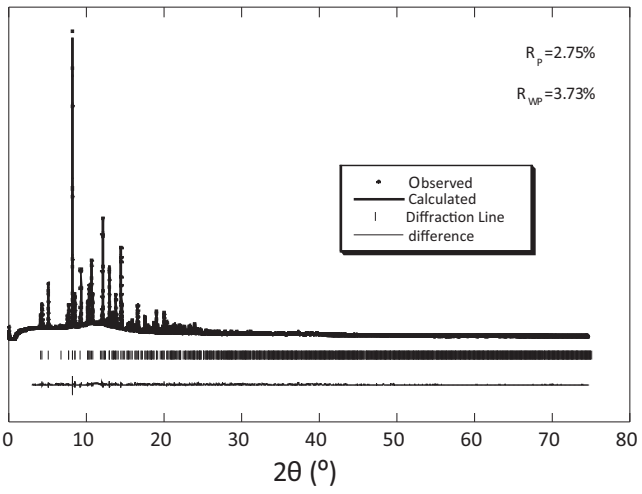

Fig. 6. Rietveld refinement plot for α-from.

The DTA curve of sesquihydrate showed broad endothermic peaks at 100 °C with 6.7% of the mass loss due to the dehydration (theoretical: 6.19%). Difference between actual mass loss and theoretical value of water content was attributed to nature of crystalline water in sesquihydrate: The occupancy of crystalline water in this crystal was disordered (Suzuki et al., 2000) and water contents fluctuated between sesquihydrate and dihydrate. In this paper, this crystalline form is referred to as sesquihydrate to exclude complexity. Above dehydration temperature, DTA curve of sesquihydrate showed endothermic peak at 127 °C and an exothermic peak at 146 °C. These peaks were considered to be a melting and recrystallization peak, respectively. After recrystallization, sesquihydrate showed endothermic peak at 220 °C that corresponded to the melting point of β-form.

XRPD was performed to elucidate crystal forms of STFX hydrates heated above melting temperature. Fig. 8 shows XRPD patterns of STFX converted from hydrates by heating in open pans and sealed pans. Crystal conversion behavior is summarized in Table 2. The

Table 2
Resultant anhydrous crystalline forms yielded by heating hydrates of sitafloxacin.

	Initial crystalline form	
Heating condition	Monohydrate	Sesquihydrate
In a open pan	α-form + β-form	β-form
In a sealed pan	α-form[a]	β-form + α-form

[a] Containing a small amount of β-form.

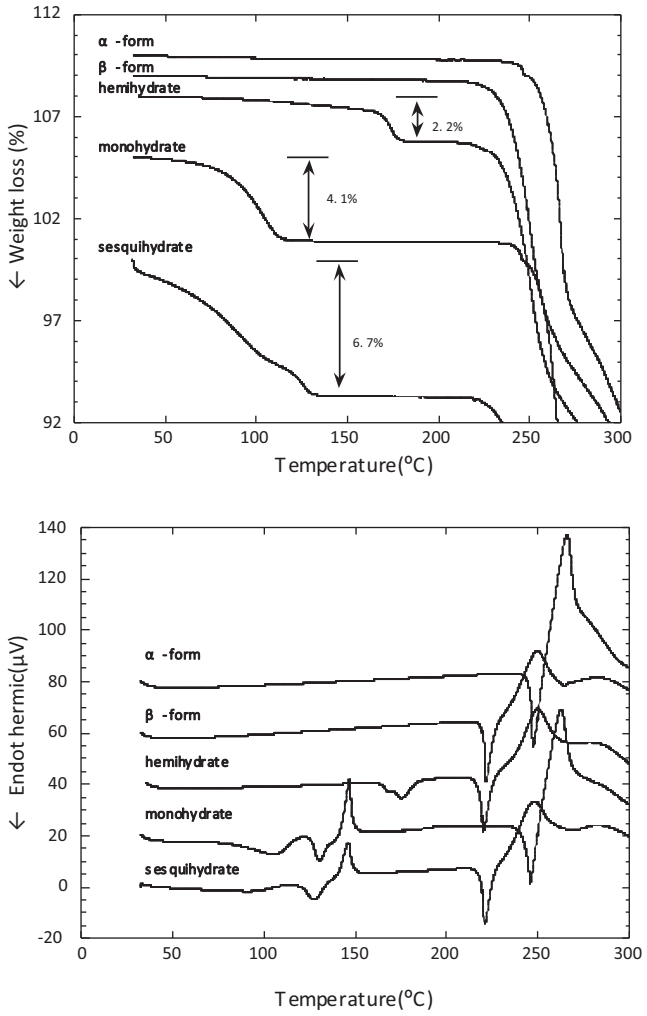

Fig. 7. TG and DTA curves of polymorphs and pseudopolymorphs of sitafloxacin.

monohydrate was converted into mixture of α-form and β-form in an open pan and into almost α-form in a sealed pan. The sesquihydrate transformed into β-form in an open pan and into mixture of β-form and α-form in a sealed pan. These results suggested that monohydrate possesses stronger tendency to transform into α-from than sesquihydrate although ratio of β-form in STFX converted from hydrates by heating seemed to be higher than expected from TG/DTA curve (Fig. 7).

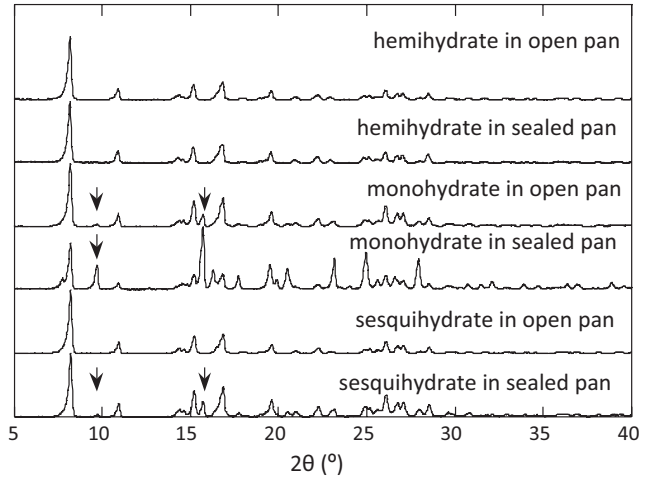

Fig. 8. XRPD patterns of sitafloxacin converted from hydrates by heating up to just above melting points in open pans and sealed pans. Arrows indicate diffraction lines characteristic for α-form.

Results of crystal structure analysis explain why monohydrate and sesquihydrate transformed into different crystal forms despite they melted and recrystallized at the same temperature range.

The conformation of quinolone ring and 5-menbered ring was retained in transformation processes where melting of hydrates and recrystallization of anhydrates occurred simultaneously.

At the beginning of these processes, dehydrated crystal forms of monohydrate and sesquihydrate remained in melted STFX and acted as seed crystal before they melted, generating α-form and β-form, respectively. Once α-form or β-form crystallized, it acted as seed crystal in recrystallization process.

It is also suggested that heating conditions affected dehydration and crystal transformation behavior of hydrates. Conversion into α-form was promoted by heating in sealed pan compared with heating in open pan when monohydrate or sesquihydrate was used as initial hydrate.

It is considered that hydrates in sealed pans transformed under the atmosphere of sufficient humidity, because water vapor generated by dehydration of hydrates was not removed as rapidly as in open pans. It is thought that STFX exist as zwitterion in an aqueous solution from dissociation constants of carboxylic group and amino group.

Like in aqueous solution, melted STFX in the sealed pan would exist as zwitterionic state stabilized by small amount of water and crystallized to α-form which has zwitterionic structure.

On the other hand, melted STFX did not contain water and formed as neutral molecule and crystallized to β-form in the open pan, because water vapor was removed rapidly by nitrogen stream.

The ionization of STFX might also inhibit to generate β-form by disrupting hydrogen bonding between COOH and C=O of pyridone. This hydrogen bonding seemed to have important roll to stabilize conformation of quinolone ring as β-form type by forming six-membered ring coplanar with quinolone ring and disruption of this hydrogen bonding made conformation of quinolone ring interconvert more easily.

This interpretation describes how the crystal transformation mechanisms of STFX were affected by heating condition.

4. Conclusion

Two anhydrates and three hydrates of STFX were characterized by infrared spectroscopy, X-ray powder diffractometry and thermal analysis. The infrared spectroscopy indicated that STFX exists as neutral molecule in β-form while exist as zwitterion in other crystal forms. The results of crystal structural analysis are consistent with this observation. Crystal structural analysis also revealed that quinolone rings of STFX have distorted planar structure and quinolone ring of STFX in α-form and monohydrate have opposite torsion to those in β-form and sesquihydrate. 5-membered ring at the 7th position in α-form and monohydrate also have different conformation to that in β-form and sesquihydrate.

DTA analysis indicated that monohydrate and sesquihydrate transformed into different anhydrous crystal forms, namely α-form and β-form, even though they transformed at the same temperature range by melting and recrystallization. The conformation of quinolone ring and 5-membered ring affected transformation behavior of hydrates. The atmosphere also had influence on transformation behavior. Presumably the water vapor generated from hydrates stabilized zwitterion that composed α-form.

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