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Elucidation of the crystal structure–physicochemical property relationship among polymorphs and hydrates of sitafloxacin, a novel fluoroquinolone antibiotic

Tetsuya Suzuki[a,*], Katsuhide Terada[b]

[a] Analytical & Quality Evaluation Research Laboratories, Pharmaceutical Technology Division, Daiichi Sankyo Co., Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan
[b] Department of Pharmaceutics, School of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

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ABSTRACT

Physicochemical properties of two anhydrates (α-form and β-form) and three hydrates (hemihydrate, monohydrate and sesquihydrate) of sitafloxacin (STFX), a novel fluoroquinolone antibiotic, were investigated and correlated with the crystal structure of each crystalline form. STFX sesquihydrate showed larger weight change between 1% and 95% relative humidities (RHs) than other crystalline forms. In the crystal of sesquihydrate, STFX molecules form a channel structure where water molecules exist. Contrary to sesquihydrate, water molecules in a monohydrate are located in well-defined and isolated crystallographic sites. The β-form exhibited the worst photostability than any other forms under the irradiation of a D65 lamp. The intramolecular hydrogen bonding in the β-form caused a red shift on the solid-state UV spectrum by prolonging the conjugation system of the quinolone ring, resulting in greater absorption of photoenergy and consequent degradation. Solubility is also affected by the crystalline structure. Standard free energy of the formation of STFX molecule in each crystalline form and/or lattice energy binding STFX molecules to retain the crystal structure might cause a difference in solubility.

1. Introduction

In order to launch new pharmaceutical products, selecting salt and crystalline forms with suitable physicochemical properties, such as hygroscopicity, solubility and stability, is an essential step as well as searching compounds with higher pharmaceutical activity and a wider safety margin.

Recently, new active pharmaceutical ingredient (API) candidates tend to have a larger molecular structure and unfavorable physicochemical properties, and the importance of solid form selection has been increasing.

Regarding formulation (Jasti et al., 1995; Kojima et al., 2008) and manufacturing (Rocco et al., 1995) processes, these form selection studies have been performed in order to choose the "best form". In addition with form selection studies, reports on the relationship between the crystal structure and physicochemical properties have been increasing (Parshad et al., 2004; Perlovich et al., 2006; Chow et al., 2008; Thirunahari et al., 2010).

Sitafloxacine(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 was recently put on the market with a trade name of Gracevit® (Daiichi Sankyo Co., Ltd.), and its antibacterial activity is significantly better than that of other fluoroquinolones (Sato et al., 1992; Touyama et al., 2006; Okuda et al., 2007). We have reported that at least five polymorphs and hydrates (anhydrate α-form, β-form, hemihydrate, monohydrate and sesquihydrate) of STFX have been found (Suzuki et al., 2010), and the crystal structural analysis has been performed for four of the crystalline forms except for hemihydrate (Suzuki et al., 2010).

In this report, several physicochemical properties, *e.g.*, hygroscopicity, dissolution behavior and photostability of STFX polymorphs and hydrates were investigated. In addition, in view of the structure–property relationships, the difference of each physicochemical property among the crystalline forms has been explained, which provides unique insights into the crystal design in order to improve physicochemical properties of APIs.

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).

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* Corresponding author. Fax: +81 3 3492 3131.
E-mail address: suzuki.tetsuya.fb@daiichisankyo.co.jp (T. Suzuki).

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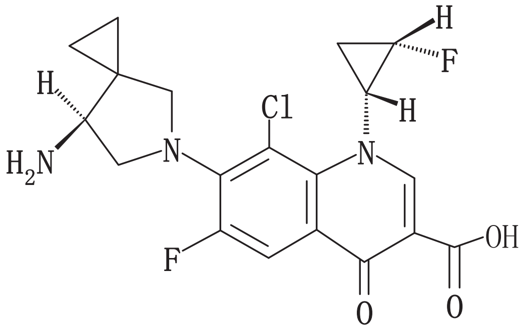

Fig. 1. Chemical structure of sitafloxacin.

2.2. Sample preparations

All samples were prepared as described in previous articles (Suzuki et al., 2010): Anhydrate α-form was obtained by recrystallizing from ethanol. Anhydrate β-form was prepared by slurring in toluene at boiling temperature for about 1 h. Hemihydrate was yielded by slurring in 1,2-propanol/water (99:1 v/v) at boiling temperature. Monohydrate was prepared by recrystallizing from methanol and stored at an ambient condition. Sesquihydrate was used without further purification.

2.3. Moisture sorption–desorption profiles

The moisture sorption–desorption analysis was carried out gravimetrically on a microbalance system (VTI SGA-CX, TA Instruments, DL, USA). After drying by purging dry nitrogen at 25 °C, weight change was monitored under stepwise increasing relative humidities at 25 °C. The equilibrium criterion for sample weight was less than 0.03% for 30 min.

2.4. Photostability

After passing through a 150 μm sieve, about 10 mg of each crystalline form was spread across 100 μL of aluminum pan and stored in a light-irradiation tester (Light-Tron LT-120, Nagano Science Co., Osaka, Japan). The lamps that were designed to produce an output similar to the D65 emission standard (D65 lamps) were used as a light source. The irradiation test was carried out at an illuminance of 3000 lux. After irradiating at 300, 600, 900 and 1200 klux h (l× h), each sample was removed from the light-irradiation tester and analyzed by HPLC under the same condition as previously reported (Araki et al., 2002).

2.5. Solid-state UV/vis spectroscopy

The diffuse reflectance solid-state UV/vis absorption spectra of each form were recorded on a UV-3700 DUV system (Shimadzu, Kyoto, Japan) equipped with an integrating sphere unit at room temperature. Each crystalline form was diluted with barium sulfate and filled into a cell with quartz window. The spectra were acquired with a 0.5 nm sampling pitch in the wavelength range from 250 to 800 nm. Barium sulfate was used as the reference sample.

2.6. Dissolution behavior

After passing through a 150 μm sieve, excess amounts of each crystalline form were added to water and mixed vigorously in a shaking water bath at 37 °C. At each sampling time, an appropriate amount of each solution was removed and filtered through a membrane filter (pore size: 0.45 μm), and then diluted with the appropriate volume of HPLC media. The resulting preparations were assayed by HPLC under the same condition as previously reported (Araki et al., 2002).

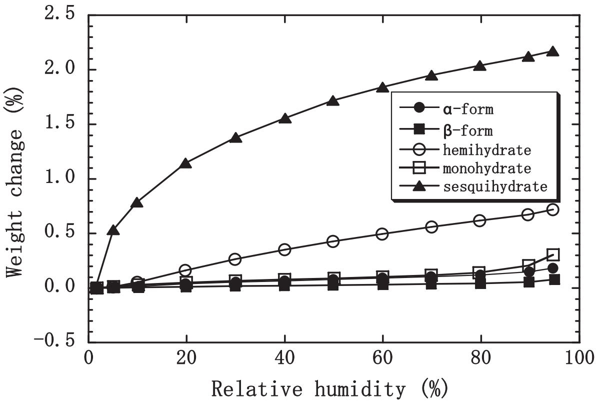

Fig. 2. Moisture sorption–desorption profiles of polymorphs and hydrates of sitafloxacin at 25 °C.

2.7. Density functional theory (DFT) calculation

Solubility of each crystalline form of STFX depends on the difference between the standard free energy of formation of each crystalline form and standard free energy of formation of STFX in an aqueous solution. The standard free energy of formation of STFX in a solution is independent of the initial crystalline form of solute. Thus, solubility depends on the standard free energy of formation of each crystalline form.

The standard free energy of the unit cell (energy/unit cell) of each crystalline form was calculated by using the program Dmol3 (Accelrys, San Diego, CA, USA) under the following conditions; Task: energy, Functional: LDA PWC, Basis set: DND, Quality: medium.

The average standard free energy of a sitafloxacin molecule (energy/mol) in each crystalline form was calculated as follows: Energy/mol = ((Energy/unit cell) − (Energy of water/unit cell))/N, where N is the number of sitafloxacin molecules in the unit cell.

The energy of water/unit cell was calculated by the same method for each unit cell of hydrate. DFT calculation was conducted after removing STFX molecules from the unit cell of hydrate.

3. Results and discussion

3.1. Moisture sorption–desorption profiles

Fig. 2 shows the moisture sorption–desorption profiles of the five forms of STFX. Sesquihydrate showed approximately 2% of weight change between 1% and 95% relative humidities (RHs). This weight change corresponds to 0.5 mol water/mol of STFX sesquihydrate (theoretical value: 2.1%).

Weight changes of hemihydrate and monohydrate were very slight. These observations showed that hemihydrate and monohydrate were kinetically stable independents of RHs. Anhydrates did not show weight changes. Crystal structures of STFX polymorphs and hydrates are shown in Fig. 3 (Suzuki et al., 2010). In sesquihydrate, STFX molecules form a channel structure where water molecules exist. The channel structure of sesquihydrate is shown in Fig. 4. There are two STFX molecules and four crystallographic sites for water molecules in an asymmetric unit and the occupancy of sites for water molecules are varied, depending on the RHs. This particular structure may cause non-stoichiometric hydration, as previously reported (Stephenson et al., 1998; Authelin, 2005). The crystal structural analysis showed that one or two water molecules are disordered in occupancy. Contrary to sesquihydrate, water molecules in a monohydrate are located in well-defined and

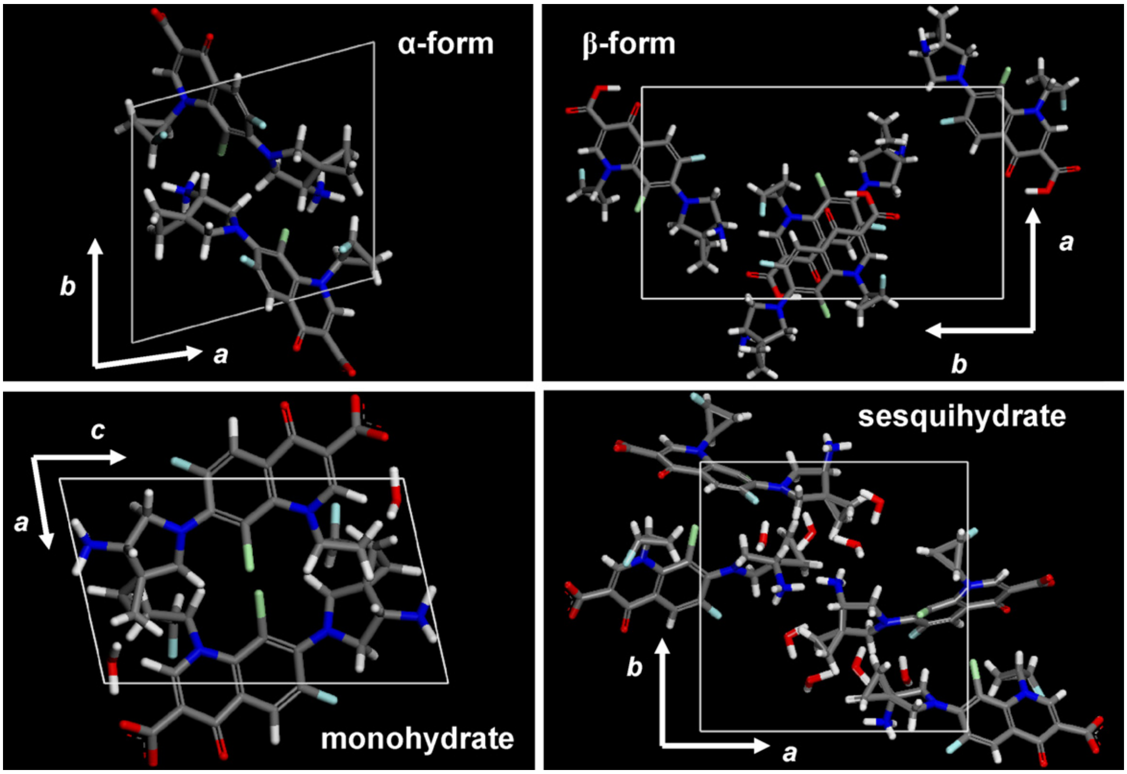

Fig. 3. Crystal structures of polymorphs and hydrates of sitafloxacin.

isolated crystallographic sites (Suzuki et al., 2010). The difference of the structure of crystallographic sites where water molecules exist causes different moisture sorption–desorption profiles between sesquihydrate and monohydrate. Unfortunately, the crystal structure of hemihydrate has not been determined.

3.2. Photostability

HPLC chromatograms of the five crystalline forms before and after irradiating at 1200 klux h with the D65 lamp are shown in Fig. 5. HPLC chromatograms before irradiation revealed that each crystalline form was pure and the amount of impurity was negligible. After irradiation, several peaks with a number of minor peaks were observed on HPLC chromatograms in addition with the peak of STFX. For each form, no peaks attributing to the degradation products were observed without light irradiation, during the testing period. This shows that STFX was decomposed by irradiating with the D65 lamp. Fig. 6 shows the time course of purity of STFX, under the irradiation of the D65 lamp. Though the HPLC chromatograms obviously show peaks of the degradation products, the decrease in purity (percent of the total area) was not steep for α-form, hemihydrate, monohydrate or sesquihydrate. On the contrary, β-form decomposed significantly faster than other crystalline forms. It is reported that the rate of photodegradation is affected by several factors, such as the surface area of a sample (Teraoka et al., 2004) and the atmosphere of the photo-irradiation

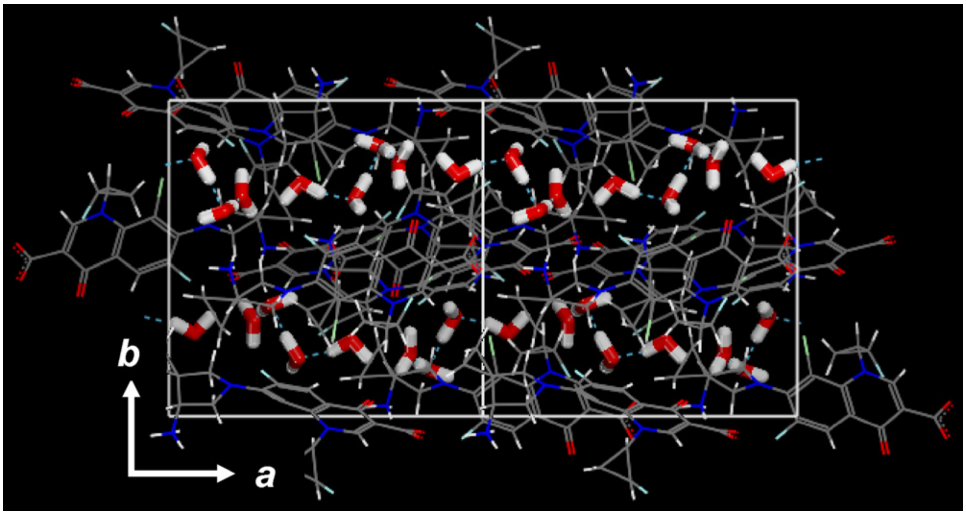

Fig. 4. Water molecules in channel structure made of STFX molecules in STFX sesquihydrate. Hydrogen bonds between water molecules are indicated by right blue doted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

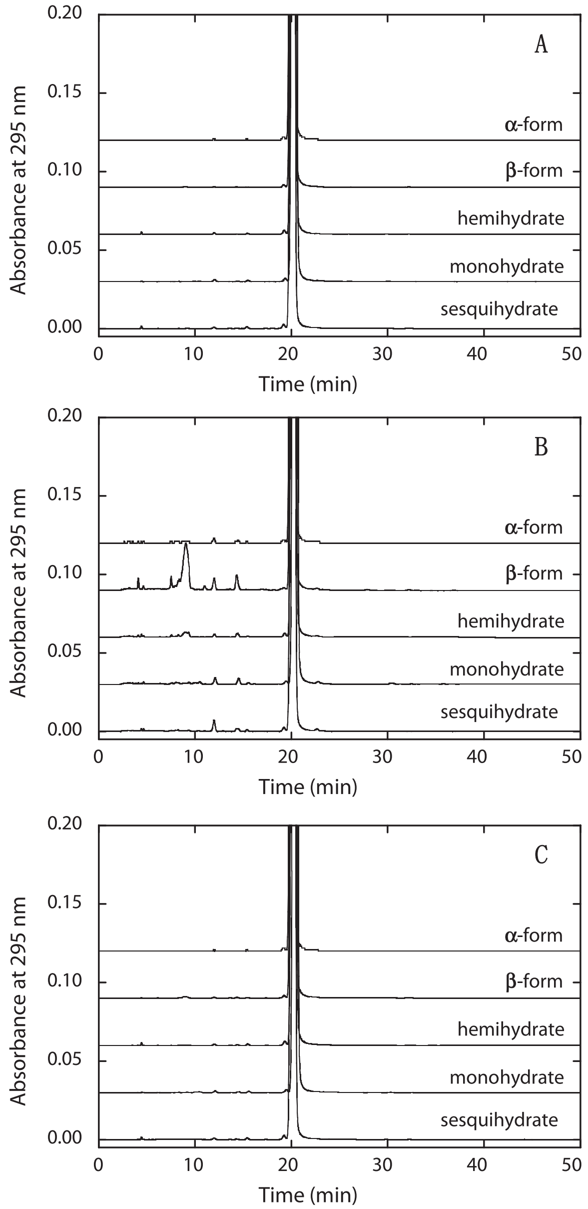

Fig. 5. HPLC chromatograms of polymorphs and hydrates of sitafloxacin before and after irradiating with the D65 lamp. (A) Before irradiation, (B) after being irradiated with the D65 lamp for 1,200,000 lux h, (C) after being stored in a test chamber without light irradiation.

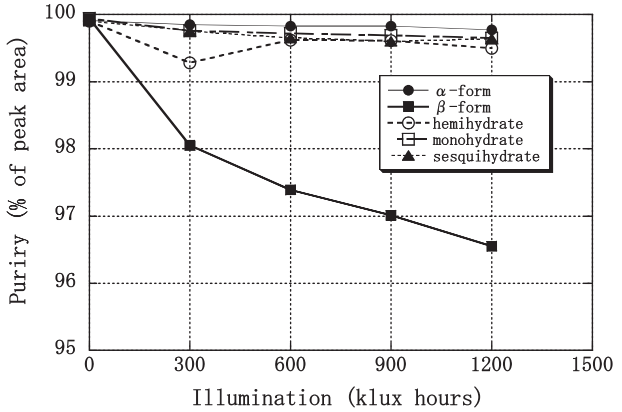

Fig. 6. Time courses of polymorphs and hydrates of sitafloxacin under irradiation of the D65 lamp.

chamber (De Villiers et al., 1992). Photomicrographs of testing samples are shown in Fig. 7. Particle sizes of β-form, monohydrate and sesquihydrate are equivalent and larger than those of α-form and hemihydrate. The effect of particle size on photostability was investigated in detail by Teraoka et al. (1999). They reported that the photodegradation rate constant of nifedipine powder depended on the particle size, and decreased with an increase in the particle size. This phenomenon will be the case with other compounds because photodegradation occurs at the surface area exposed to light (De Villiers et al., 1993; Teraoka et al., 1999; Kojima et al., 2007). In our study, photodegradation of β-form monohydrate and sesquihydrate might be underestimated. However, β-form exhibited the fastest degradation. This shows β-form is significantly less stable against light than other crystalline forms.

It is reported that the solid-state photostability of polymorphs has correlations with spectroscopic properties (Kojima et al., 2007) and the heat of fusion (Teraoka et al., 2004; Matsuda et al., 1994). Spectroscopic properties have a close relation with energy absorption from irradiated light, and heat of fusion seems to affect the rate of degradation after it is excited by absorbing light.

3.3. *Solid-state UV/vis spectroscopy*

It is reported that STFX degraded the wavelength dependently after being exposed to light of various wavelengths at the same energy in a solution, and that the degradation rate and UV spectrum of STFX well correlated (Araki et al., 2002). This suggests, at least in a solution, that absorbing photon energy is a critical step of photodegradation. It was also reported that the difference of photostability of tamoxifen citrate polymorphs could be attributed to the difference of spectroscopic properties. In other words, the light sensitive crystalline forms showed stronger absorbance at a wavelength region of irradiation light (Kojima et al., 2007).

Fig. 8 shows the solid-state UV/vis absorption spectrum of each form overlaid with the irradiation spectrum of the D65 lamp. In this figure, the Kubelka–Munk function (KMF) of each form was normalized so that each spectrum could be compared qualitatively. The D65 lamp showed maximum irradiation peaks at around 433 nm, 409 nm and 367 nm with relative intensity of 100%, 43% and 28%. The irradiation energy of the D65 lamp at below 300 nm was negligible.

The most meaningful difference among the spectra of the five crystalline forms of STFX is that the maximum wavelength below STFX absorbs photoenergy: the normalized KMF of α-form decreased steeply above approximately 360 nm, that of hemihydrate, monohydrate and sesquihydrate also fell above approximately 370 nm, 390 nm and 380 nm, respectively. Different from these four crystalline forms, β-form showed a large normalized KMF value at the widest wavelength range between 250 nm and 410 nm, that included two maxima of irradiation spectrum of the D65 lamp. The difference of the KMF value around 400 nm would cause a large difference in absorption of photoenergy, from the D65 lamp and degradation upon exposure to the light of the D65 lamp.

In our previous investigation, it was proven that STFX exists in a neutral molecule only in the β-form and exists in zwitterions in other crystal forms (Suzuki et al., 2010). It is also reported that STFX formed an intramolecular hydrogen bond between the carboxyl group and the carbonyl moiety (Yamazaki and Suzuki, 1998; Suzuki et al., 2010). This hydrogen bonding modified the structure of major chromophore of STFX. Namely, the conjugation system was prolonged and a new cyclic structure was formed by hydrogen bonding and STFX may act like a compound of the three-cyclic ring system (Fig. 9).

This modification on major chromophore would cause a difference of the solid-state UV/vis spectra and different stability under

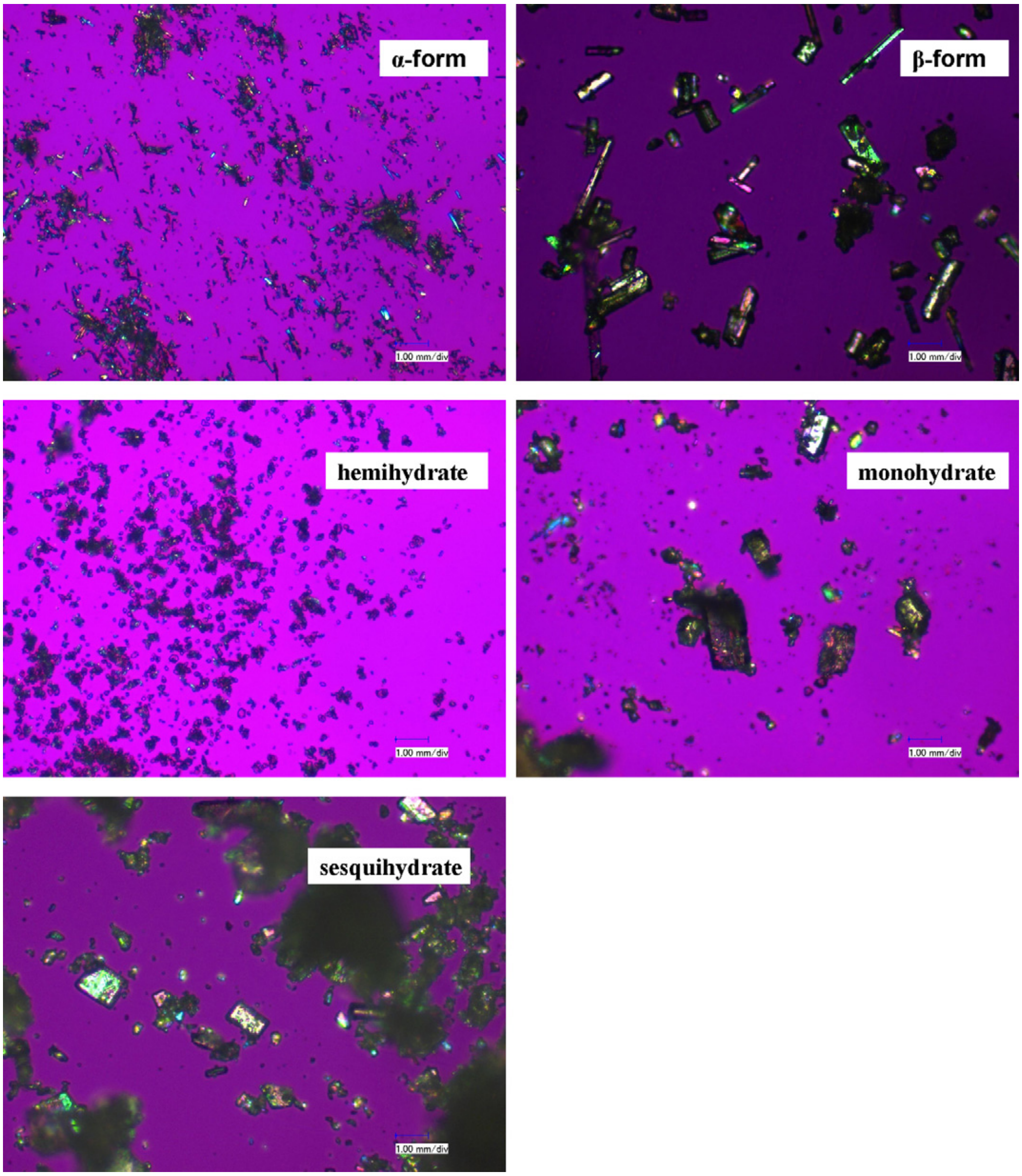

Fig. 7. Photomicrographs of polymorphs and hydrates of sitafloxacin used in photostability testing.

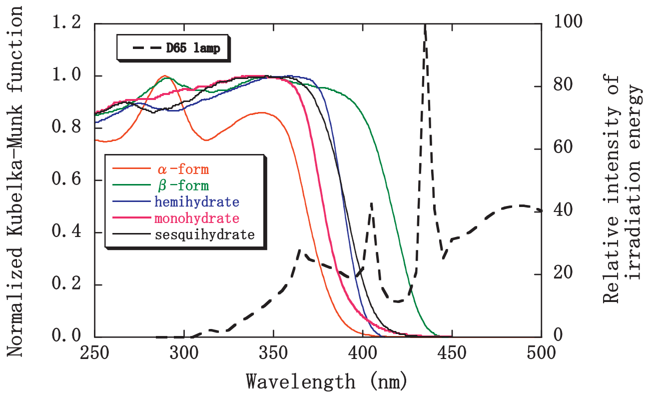

Fig. 8. Solid-state ultraviolet–visible absorption spectrum of polymorphs and hydrates of sitafloxacin overlaid with the irradiation spectrum of the D-65 lamp.

irradiation of the D65 lamp. Of course, as previously reported, the solid-state UV/vis spectra and photostability would also depend on the arrangement of the molecule in the crystal (Mizuguchi et al., 1995; Kojima et al., 2007).

3.4. Dissolution behavior

Dissolution patterns of polymorphs and hydrates of STFX in water at 37 °C are depicted in Fig. 10. For the α-form, the β-form and monohydrate, the concentration in bulk liquid increased rapidly, and reached the maximum value within 10 min. After that, the concentrations then fell gradually to the same as solubility of sesquihydrate. This observation suggests that these forms were converted into sesquihydrate. Different from these three crystalline forms, in the case of hemihydrate and sesquihydrate, concentrations of STFX reached constant values after 10 min and no further significant change was detected. These show that hemihydrate and sesquihydrate were stable during the dissolution test. Fig. 11 shows XRPD patterns of polymorphs and hydrates of STFX before and after the dissolution test. It was confirmed by XRPD that α-form, β-form

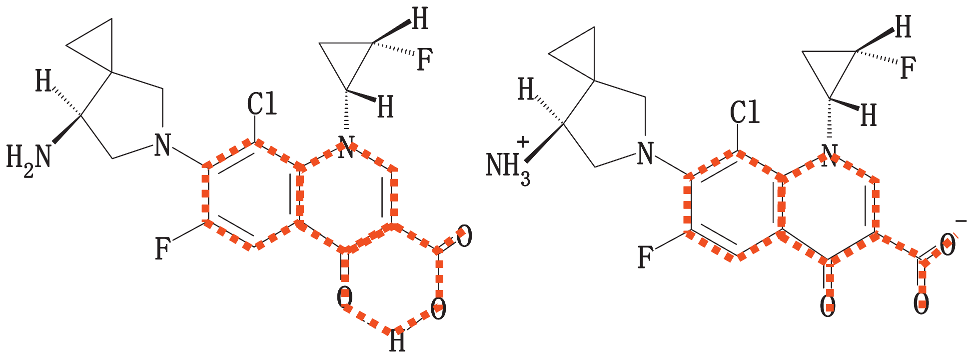

Fig. 9. Molecular structure and estimated hyperconjugation system of STFX. Left: in the β-form, right: in the α-form, hemihydrate, monohydrate and sesquihydrate. The hyperconjugation systems of STFX are shown as red dotted lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

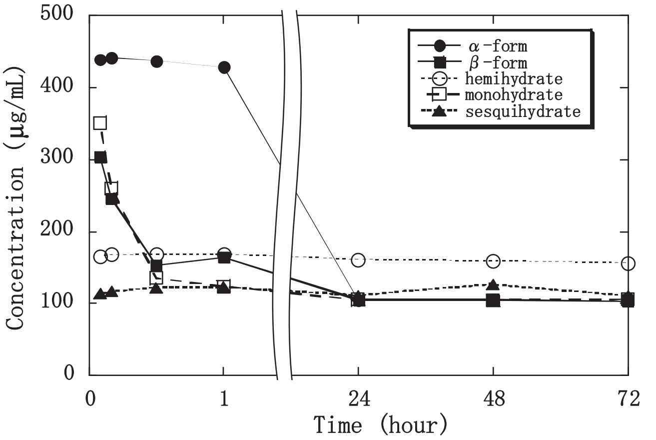

Fig. 10. Dissolution behavior of STFX polymorphs and hydrates into water at 37 °C.

and monohydrate transformed into sesquihydrate under the dissolution test condition, though hemihydrate and sesquihydrate were stable during the dissolution test. In general, the stable form shows the lowest solubility. From this point view, sesquihydrate is the stable form and the other four crystalline forms are metastable under the condition of the dissolution test. The reason why hemihydrate did not convert into sesquihydrate, was not clear. However, it is presumably because solubility of hemihydrate is not high enough to yield labile supersaturated solution, where spontaneous crystallization of sesquihydrate occurs. Dissolution of hemihydrate might give metastable supersaturated solution where spontaneous crystallization of sesquihydrate is improbable (Mullin, 2001).

The maximum concentration of STFX was about 4.0, 2.8, 1.6 and 3.0 times higher than that of sesquihydrate for the α-form, the β-form, hemihydrate and monohydrate, respectively.

It is worth emphasizing that the monohydrate showed higher maximum concentration than the β-form, nevertheless, hydrates of APIs usually show lower solubility than those of anhydrates (Ledwidge et al., 1996; Li et al., 1996; Ito et al., 1997). It is also noticeable that the α-form showed significantly higher maximum concentration than the β-form.

Fig. 12 shows STXF molecules in several crystals (Suzuki et al., 2010).

The quinolone ring of STFX did not take planner conformation. The distortion of quinolone ring would arise from steric hindrance between the chorine atom at the 8th position and cyclopropyl moiety at the 1st position. The chlorine atoms in β-form and seseuihydrate go upward from the averaged plane of quinolone ring and cyclopropyl moieties go downward (β-type). Distortion of the quinolone ring of STFX molecules in the α-form and monohydrate is opposite to those observed in the β-form and sesquihydrate (α-type). The crystalline forms, those containing α-type STFX molecule (α-from and monohydrate), showed a higher maximum concentration than those containing β-type STFX molecule (β-form and sesquihydrate). This implies that the distortion at the quinolone ring may affect the solubility. However, the mechanism has not been proved.

3.5. Energy calculation by density functional theory

The standard free energy of each crystalline form calculated by DFT calculation is shown in Table 1. The standard free energy of

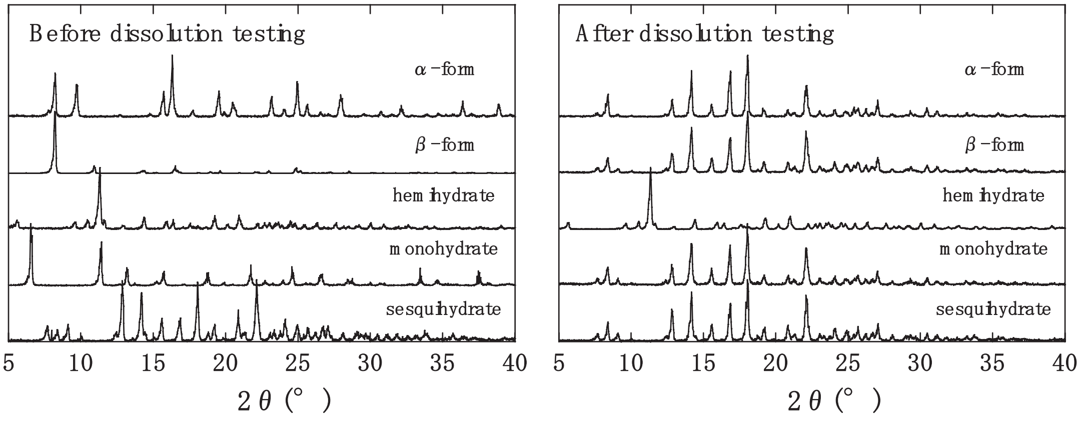

Fig. 11. XRPD patterns of polymorphs and hydrates of sitafloxacin before and after dissolution testing.

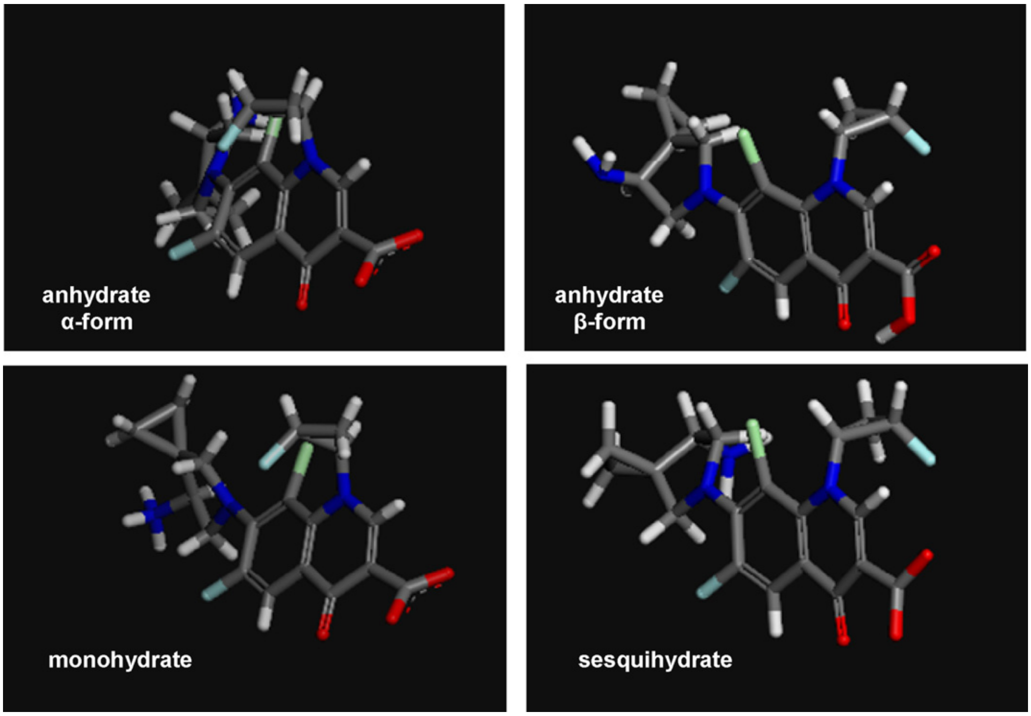

Fig. 12. Conformation of sitafloxacin in several crystalline forms. The quinolone ring in α-form and monohydrate is distorted so that the cyclopropane ring comes upward from the quinolone ring and the chlorine atom goes downward from the quinolone ring in this figure. The quinolone ring in β-form and sesquihydrate is distorted oppositely.

Table 1
Standard energy of formation of STFX polymorphs and hydrates calculated by DFT calculation.

Crystalline forms	Standard energy of formation of crystal (kJ/(NA × unit cell))	Standard energy of formation of water molecule (kJ/(NA × unit cell))	Number of STFX molecules in the unit cell	Standard energy of formation/STFX molecule in the crystal (kJ/mol)
α-Form	−48,702	–	2	−24,351.0
β-Form	−97,494	–	4	−24,373.6
Monohydrate	−51,104	−1925	2	−24,589.2
Sesquihydrate	−213,546	−16,190	8	−24,669.5

NA: Avogadro number.

formation of α-form and β-form agreed well with the order of solubility of each form. The standard free energy of formation of hydrates seemed lower than expected from solubility. This discordance arises from the difficulty of estimating the contribution of water of hydration on the standard free energy of formation of unit cell. Improvements of the calculation method and the following experiments on other compounds would solve this matter.

4. Conclusion

Physicochemical properties of two anhydrates and three hydrates of STFX were investigated and correlated with the crystal structure of each form. The moisture sorption–desorption profile has a close relationship with the structure of crystallographic sites where water molecules are located. The intramolecular hydrogen bond between the carboxyl group and the carbonyl group in the β-form, caused a red shift of the solid-state UV/vis spectrum edge of STFX, by modifying the structure of a major chromophore. As a result, the β-form exhibited significant absorption at a peak wavelength of irradiation of the D65 lamp around 409 nm, and absorbed much more energy than other crystalline forms. This would be the reason why the photostability of the β-form is worse than the other forms. The crystal structure also affected solubility presumably through the standard free energy of the formation of STFX molecule, and/or lattice energy binding STFX molecules to retain the crystal structure.

This report was analyzed to elucidate the relationship between physicochemical properties and crystal structure of APIs, and to promote the crystal design for the improvement of physicochemical properties.

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