Characterization of melt-quenched and milled amorphous solids of gatifloxacin

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ABSTRACT

The objectives of this study were to characterize and investigate the differences in amorphous states of gatifloxacin. We prepared two types of gatifloxacin amorphous solids coded as M and MQ using milling and melt-quenching methods, respectively. The amorphous solids were characterized via X-ray diffraction (XRD), nonisothermal differential scanning calorimetry (DSC) and time-resolved near-infrared (NIR) spectroscopy. Both the solids displayed halo XRD patterns, the characteristic of amorphous solids; however, in the non-isothermal DSC profiles, these amorphous solids were distinguished by their crystallization and melting temperatures. The Kissinger–Akahira–Sunose plots of non-isothermal crystallization temperatures at various heating rates indicated a lower activation energy of crystallization for the amorphous solid M than that of MQ. These results support the differentiation between two amorphous states with different physical and chemical properties.

KEYWORDS

Amorphous solid; gatifloxacin; non-isothermal DSC; time-resolved near-infrared spectroscopy; X-ray diffraction

Introduction

Further understanding of the crystal polymorphism of a pharmaceutical compound is critical for the optimization of its bioavailability and therapeutic levels in patients. Differences in the crystal forms induce significant changes in stability depending on the thermomechanical history of the material. The maintenance of crystal stability is essential to ensure the stability and secure dissolution characteristics of pharmaceutical products. In the concept of quality by design (QbD), process control of pharmaceutical manufacturing is carried out via real-time monitoring of critical material attributes (CMA) and controlling critical process parameters (CPP). Polymorphism of active ingredients is one of the most important CMA contributing to the critical quality attributes (CQA) of products. Thus, it is necessary to recognize the CPP changes in polymorphism.

A number of studies have investigated crystal polymorphisms, characteristics of amorphous solids and the related thermodynamic properties of various drug compounds, such as indomethacin, carbamazepine, imidafenac and others. Most of these studies used analytical approaches based on X-ray diffraction (XRD), differential scanning calorimetry (DSC) and vibrational spectroscopy. The objective of these investigations was to characterize and classify crystal structures and thermodynamic stability of polymorphic and amorphous forms.

An alternative method to control crystal polymorphism is to improve the solubility of materials with low solubility in water, e.g. to prepare an amorphous form of the material. Prior studies have shown that amorphous solids possessed different thermodynamic energy, stability and different terahertz spectra. The authors concluded that amorphous solids can be distinguished by nanostructures in an irregular network. Thus, when a low number of clusters, such as dimers or trimers, irregularly disperse in the amorphous state, the physical and thermodynamic properties are different depending on the cluster level. It is known that the mechanical processing of solid materials induces phase change between the crystalline and amorphous states. Metals and alloys have been reported to have mechanically induced phase changes, followed by inorganic and organic materials. Many authors reported that the milling of crystals might lead to an amorphous state. However, the milling of solids resulted in the micronization of the crystals, which were then too small to be analyzed by XRD. Therefore, the crystal structure was changed to a disordered state of micro-aggregated crystals by mechanical stress. This micronization increases the surface area of solids, thus, promoting interactions between API and co-former, which lead to the formation of a cocrystal structure. Hence, the cocrystal obtained through a co-milling method presents randomness in its structure.

Polymorphism has been studied for more than 34 years, and there are a number of reports regarding the polymorphism of amorphous ice, SiO2 and other species. The polyamorphic forms of ice, defined by Mishima as low-density and high-density amorphous, are prepared under high pressure and a first-order transition between two amorphous phases of ice is induced by pressure. The significance of polymorphism in pharmaceutical sciences was described by Hancock, who suggested that the term "pseudo-polymorphism" may be most appropriate for the amorphous solids departed from an equilibrium state. In the non-equilibrium state of amorphous solids, it is possible to isolate amorphous solids with distinct physical and chemical properties. Pseudo-polymorphism is more likely to occur in the pharmaceutical manufacturing process or during storage, depending on the conditions; thus it is important to isolate the amorphous states based on their properties.

The concept of polymorphism or pseudo-polymorphism and its significance in pharmaceutical and material sciences has been described previously. However, the most important issue for the industry is not only to define a material as being in a...
polyamorphic form, but also to understand the characteristics of the material to be processed. In the present study, we try to distinguish the differences in amorphous states of gatifloxacin (GFLX, Figure 1) by investigating the crystallization mechanisms using XRD, non-isothermal DSC and near-infrared (NIR) spectroscopy. The model pharmaceutical ingredient GFLX is an antibiotic of the fourth-generation fluoroquinolone family. Several hydrate and anhydrous crystalline polymorphs of GFLX have been identified. Moreover, there are several quinolone drugs such as levofloxacin and norfloxacin. The drug–drug interactions often become problematic for the quinolone family. GFLX has already been withdrawn because of hypoglycemia and hyperglycemia; however, its recrystallization property will be useful to understand the properties of the aforementioned interactions.

Methods

Sample preparation

Bulk GFLX sesquihydrate (PubChem CID: 5282384) was obtained from Kyorin Pharmaceutical Co., Ltd. (Tokyo, Japan). The bulk powder was dehydrated to obtain anhydrous crystals by heating at 110 °C. The amorphous form of M was prepared by milling 5 g of the anhydrous crystal using a centrifugal ball mill (Fritsch, Idar-Oberstein, Germany) with a 1 L agate pot and 15 agate balls for 3 h at 200 and 300 rpm of rotational speed. The amorphous form of MQ was prepared by melting the bulk crystal at 200 °C and quenching the melt with liquid nitrogen. The quenched solid was then dried under reduced pressure for 1 h at room temperature. The dried solid was broken into fine particles with an agate mortar, and the sample powder was dried once more under reduced pressure for 24 h at room temperature.

Measurements

XRD patterns of the amorphous and recrystallized solids were collected using an X-ray diffractometer (RINT-ULTIMA III, Rigaku, Tokyo, Japan). The X-ray source was a Cu-Kα at 40 kV and 40 mA. The diffracted X-ray signal was scanned between 5 and 35° in 2θ steps of 0.02°.

Non-isothermal DSC profiles of the two amorphous forms were determined using a differential scanning calorimeter (DSC-8230, Rigaku) at various heating rates of 1.0, 2.0, 2.5, 5.0, 7.5, 10, 15 °C/min. Approximately, 5 mg of sample was used for all measurements. An aluminum pan and Al2O3 were used as the sample pan and the reference, respectively.

Diffuse reflectance NIR spectra were collected using a Fourier transform-NIR spectrometer (MPA, Bruker Optics, Ettlingen, Germany) with an integrating sphere attachment and a PbS photoconductive detector. NIR spectra were continuously collected at intervals of 10 s in the range of 9000–4000 cm⁻¹ with 4 cm⁻¹ spectral resolution and accumulated with eight scans. The time-resolved NIR spectra were measured under controlled temperature using a transparent glass heater (BLAST, Kanagawa, Japan).

Results and discussion

The prepared solids, M and MQ, were examined to confirm if they were amorphous solids. In Figure 2, XRD patterns of the solid samples and corresponding thermally treated solids are shown. Under the condition of milling at 200 rpm, the diffraction peaks caused by crystalline solids were still observed even if milled for 3 h. When the solid was milled at 300 rpm, the typical halo pattern of amorphous solids was observed by milling for more than 3 h. The prepared MQ solid was also confirmed to be an amorphous solid by its halo pattern. Both of the amorphous solids crystallized to a similar crystalline structure upon heating.

The crystalline solid formed by amorphous M displayed several characteristic diffraction peaks, such as 7.84°, 10.3°, 12.9°, 13.68°, 19.76° and 25.98°. Diffraction peaks of the other crystalline solid formed by amorphous MQ appeared at slightly smaller diffraction angles than that of the former crystalline solid, e.g. 7.76°, 10.24°, 12.82°, 13.64°, 19.6° and 25.8°. According to Bragg’s law, the smaller the diffraction angle, the longer the distance that exists between the diffraction planes, and there is a larger alignment of atoms; thus, the density of the solid is represented by the diffraction angle. Both diffraction patterns seemed to represent similar crystalline structures; however, there were slight differences between the two crystalline solids. Additionally, although the crystal produced by amorphous M displayed single peaks at 20.58° and 23.78°, doublet peaks were observed at the corresponding diffraction angle of the other crystalline solid. These doublet peaks may indicate spatial heterogeneity in the crystal structure.

Figure 3 shows the non-isothermal DSC profiles of the amorphous solids M and MQ. The DSC profile of the initial crystal of GFLX is attached as a supplemental data, Figure S1. Both of the amorphous solids displayed an exothermic peak of crystallization following a baseline shift owing to the glass transition, and an endothermic peak of melting appeared. The characteristic temperatures of glass transition (Tg) crystallization and melting increased with increase in heating rate.

The glass transition of amorphous solids was unclear, but it seemed to be in the broad range of 80–100 °C and 40–70 °C for MQ and M, respectively. As the amorphous solid MQ was prepared...
by heating at 200 °C, the higher \( T_g \) resulted from the thermal history.

The following discussed exothermic events of forms MQ and M were observed at 110–120 °C and 90–110 °C, respectively. Both the glass transition and the crystallization temperatures of amorphous MQ were higher than that of amorphous M; thus, the amorphous state of MQ was more stable than the other amorphous state of M. In contrast, the crystallization of the amorphous form of MQ began with a shorter period of supercooled state than that of amorphous M. As the mobility of molecules increased with increase in temperature, the glass transition at higher temperatures apparently led to the fast crystallization. In other words, the slow diffusion at lower temperatures resulted in the long-term supercooled state and the following slow crystal growth.

The recrystallized solids from amorphous M and MQ showed slight differences in their diffraction angles as shown in Figure 2. However, the melting temperatures of the solid from amorphous M were 181–193 °C and those of the solid from amorphous MQ were 161–175 °C at each heating rate. The higher melting temperature resulted from the greater stability of the crystalline form of M; thus the difference in the initial amorphous states of M and MQ may strongly contribute to the recrystallization.

According to a previous report, the amorphous state induced by chemical stress, such as milling, includes clusters that are too small to be detected by XRD measurements. The mobility of molecules increased with increase in temperature, the glass transition at higher temperatures apparently led to the fast crystallization. In other words, the slow diffusion at lower temperatures resulted in the long-term supercooled state and the following slow crystal growth.

The analytical method provides the activation energy of the crystal growth process. The Kissinger–Akahira–Sunose plot is shown in Figure 4. The activation energy was determined as the slope of the linear relation, and 162 kJ mol\(^{-1}\) and 248 kJ mol\(^{-1}\) were obtained as the activation energy of M and MQ amorphous solids, respectively. The lower activation energy of amorphous form of M is due to the preexistent nuclei being a micro cluster. Moreover, the distance between molecules in the amorphous solid of M was shown to be shorter than that of the amorphous solid of MQ by the XRD patterns as shown in Figure 2.

For investigating the interaction between molecules in the amorphous solids, time-resolved NIR spectra of the amorphous solid form of M at 85 °C at a collection rate of 0.1 s\(^{-1}\). The solid black line indicates the initial spectrum, and variations in the spectra are indicated in gray within the broken line. Bands assignments of (i)–(iv) are listed in Table 1.
observed in the spectra because of the presence of CH, NH and CO groups. The vibrational modes and their assignments are summarized in Table 1. The CH bands in the ranges of 7500–7000 cm\(^{-1}\) and 6200–5700 cm\(^{-1}\) denoted a small change because of the baseline shift.

Two bands at 6503 and 6412 cm\(^{-1}\) were assigned to the first overtone of the NH stretching mode because of free and associated NH groups, respectively. In both the amorphous forms of M and MQ, the band at 6503 cm\(^{-1}\) increased; thus, the hydrogen bonds between NH groups dissociated upon changing to the supercooled state.

In Figure 6, a characteristic band in the range of 5400–4900 cm\(^{-1}\) is observed for the amorphous form of MQ. This band was assigned to the C=O stretching mode of the carboxyl group and disappeared in the supercooled state. Similarly, the broad band around 7000–6600 cm\(^{-1}\) was also reduced and was assigned to the first overtone of the OH stretching mode of the carboxyl group. The disappearance of these bands indicated that a cyclic dimer was formed between the carboxyl groups, as the cyclic dimer has a symmetric structure, and the dipole moment of carboxyl groups is unchanged by its stretching. Thus, C=O and OH stretching modes were IR active in the monomer, but IR inactive in the cyclic dimer\(^{26}\). In the amorphous form of M, the cyclic dimer was already formed, and the dimer structure was maintained in the supercooled state. Thus, the critical difference between the amorphous forms of M and MQ is that M consists of dimers, whereas MQ consists of monomers.

According to the XRD results of the crystalline solids, it was denoted that the distance of molecules was closer in the crystal structure of form M than that of form MQ. The non-isothermal DSC and the Kissinger–Akahira–Sunose plots resulted in a lower activation energy for changing to the crystal state compared with that of the other amorphous solid. The energy state of the milled amorphous solid clearly departed from that of the other amorphous solid.

**Figure 6.** Time-resolved NIR spectra of the amorphous solid form of MQ at 95 ºC at a collection rate of 0.1 s\(^{-1}\). The solid black line indicates the initial spectrum, and variations in the spectra are indicated in gray within the broken line. Bands assignments of (i)–(iv) are listed in Table 1.

**Table 1.** NIR band assignments of spectra shown in Figures 5 and 6.

| Wavenumber (cm\(^{-1}\)) | Component | Assignment |
|--------------------------|------------|------------|
| (i) 5300–5000            | C=O        | Second overtone of CO stretching |
| (ii) 6200–5600           | CH, alkene | First overtone of CH or C=C stretching |
| (iii) 6600–6300          | NH         | First overtone of NH stretching |
| (iv) 7000–6600           | OH         | First overtone of OH stretching |
| (v) 7300–7000            | CH         | Combination between first overtone of CH stretching and CH bending |

Furthermore, the results from NIR spectroscopy showed that the amorphous form of M involved clusters, and they supported the interpretation of the results from XRD and non-isothermal DSC measurements. Descamps et al. performed cryomilling of glassy indomethacin and also reported that milling placed the glass into a very high energy state\(^{27}\). Finally, we suggest that both the states of forms M and MQ are amorphous solids; however, these states are in distinct energy states.

**Conclusion**

Differences in the two amorphous states of GFLX were investigated via XRD, non-isothermal DSC and time-resolved NIR spectroscopic measurements. These measurements distinguished between amorphous solids prepared by melt-quenching and milling methods. In the amorphous solid prepared by the milling method, GFLX molecules were closely attached as dimer structures via hydrogen bonds between carboxyl groups. The amorphous solid had a low activation energy when changing to the crystal state compared with that of the other amorphous solid. The energy state of the milled amorphous solid clearly departed from that of the other amorphous solid.

**Disclosure statement**

The authors declare that they have no conflicts of interest to disclose.

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