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Research Paper

Quantitative crystallinity determination for E1010, a novel carbapenem antibiotic, using differential scanning calorimetry

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Keywords
crystallinity; differential scanning calorimetry; enthalpy change; hydrate; quantitative determination

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Abstract

Objectives The objective of this study was to develop a quantitative crystallinity analysis method for the bulk drug of E1010 ((+)-(4*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-[(2*S*,4*S*)-2-[(*R*)-1-hydroxy-1-[(*R*)-pyrrolidin-3 -yl]methyl] pyrrolidin-4-yl]thio-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrochloride), a novel carbapenem antibiotic.

Methods X-ray analyses, thermal analyses and hygroscopicity measurements were used to elucidate the crystal structure and the solid state properties. To develop a quantitative method for the crystallinity of E1010 bulk drug, the relationship between enthalpy change obtained by differential scanning calorimetry (DSC) and crystalline form ratio was investigated.

Key findings E1010 bulk drug was found to exist in a crystalline trihydrate formed in two layers, i.e. a layer of E1010 free form, and a layer consisting of chloride ions and water molecules. The thermal analysis showed an endothermic peak derived from dehydration with the loss of crystal lattices at around 100°C as an onset. The enthalpy change value for the endothermic peak correlated well with crystalline content in binary physical mixtures of the crystalline trihydrate and the amorphous form. In addition, for nine lots of the bulk drug, a positive correlation between the enthalpy change and chemical stability in the solid state was observed.

Conclusions This quantitative analysis of crystallinity using DSC could be applicable for the quality control of the bulk drug to detect variability among manufacturing batches and to estimate the chemical stability of partially amorphous samples.

Introduction

The degree of crystallinity or amorphism in pharmaceutical materials is recognized as a critical factor that may affect chemical stability and dosage form performance.[1–4] For some pharmaceuticals, e.g. carbapenem antibiotics such as meropenem and imipenem, a low degree of crystallinity correlates with poorer chemical stability.[5–7] Consequently, it is important to monitor the extent of crystallinity during the lifetime of a pharmaceutical product, from bulk material scale-up, formulation development, manufacturing, and its intended shelf-life. The quality of active pharmaceutical ingredients is typically monitored by physicochemical properties, because batch-to-batch variability often occurs due to mixed amorphous forms.[8,9] Therefore, quantitative analyses to evaluate degrees of crystallinity or quantification of the amorphous content are urgently needed in the drug development field. A number of methods including X-ray powder diffraction (XRD), water sorption, isothermal microcalorimetry, solution calorimetry, infrared (IR) spectroscopy, Fourier transform (FT)-Raman spectroscopy, near infrared (NIR) spectroscopy, and solid state nuclear magnetic resonance (NMR) spectroscopy have been applied to quantify the amorphous content of predominantly crystalline powders.[10–28]

The bulk drug of E1010, a novel carbapenem antibiotic that exhibits a broad antibacterial spectrum and high anti-pseudomonal activity, exists as a crystalline hydrate.[29,30] Since E1010 amorphous form prepared by freeze-drying techniques is much less chemically stable than the crystalline hydrate, it is imperative to determine the amount of the amorphous form in the bulk drug for further development.[31]

In this study, the crystal structure of E1010 bulk drug, and its physical and thermal properties have been investigated. A quantitative analysis method for the degree of crystallinity has been developed using differential scanning calorimetry (DSC). In addition, the enthalpy changes obtained by DSC have been studied in detail to estimate the chemical stability of the bulk drug in the solid state for quality control.

Materials and Methods

Materials and reagents

E1010 (+)-(4*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-[(2*S*,4*S*)-2-[(*R*)-1-hydroxy-1-[(*R*)-pyrrolidin-3 -yl]methyl]pyrrolidin-4-yl]thio-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrochloride, was synthesized by Eisai Company, Ltd. E1010 bulk drug was crystallized from 2-propanol/water (4 : 1, v/v) solution, in which a 1.25-fold volume of 2-propanol was dropped into a 10% (w/v) solution of E1010 in water at room temperature and then cooled resulting in crystallization. A further 2.75-fold volume of 2-propanol was dropped into the solution to complete crystallization. The solvents used in this study were either HPLC grade or special grade. All other chemicals were of analytical grade, and the water used was filtered through a Milli-Q Water Purification System (Millipore, Bedford, MA, USA) before use.

Preparation of physical mixtures

The amorphous material was prepared by lyophilization of E1010 aqueous solution (10 mg/ml). Physical mixtures containing amorphous E1010 from 0% to 70% (w/w) in the presence of the crystalline E1010 were prepared by carefully weighing out quantities of each material on an analytical balance and mixing them in an agate mortar with a pestle under conditions where the relative humidity (RH) was less than 40%.

X-ray powder diffraction

The XRD pattern of E1010 bulk drug was measured with a Rigaku RINT-2500 Ultrax18 X-ray diffractometer (Rigaku, Tokyo, Japan), using CuKα1 radiation with a wavelength of 1.54178 Å at 40 kV and 200 mA. A sample was packed into a copper holder, and the instrument was operated in the fixed time mode over the 2θ range of 15.7–26.9°, where the bulk drug has the typical diffraction peaks. Variable temperature XRD analysis was also carried out under the same measurement conditions at temperatures ranging from 40 to 140°C. The solid sample was heated to the first target temperature at the heating rate of 10°C/min. After the XRD analysis was completed, it was heated to upper target temperatures in a stepwise manner and the XRD pattern at each temperature was obtained.

Single crystal X-ray analysis

E1010 single crystals were prepared through a sitting-drop vapour diffusion method at room temperature using 2-propanol/water (3 : 2, v/v) as the reservoir solution. The droplet consisted of E1010 aqueous solution (250 mg/ml, 3 μl) and the reservoir solution (3 μl). Crystallization occurred within a day, and then the single crystal analysis was conducted with a four-circle X-ray diffractometer AFC7R (Rigaku, Tokyo, Japan), using CuKα1 radiation with a wavelength of 1.54178 Å and a rotating anode generator at 23°C. The structure was solved by direct methods and the structure refinement was performed by the full-matrix least-squares method. The refinement model for E1010 has all the non-hydrogen atoms included with anisotropic thermal displacement parameters and the hydrogens included at their calculated positions (assuming appropriate hybridization and a distance of 0.95 Å). The final reliability factor (*R*) and weighed reliability factor (*Rw*) values for E1010 observed reflections were 0.040 and 0.059, respectively.

Hygroscopicity measurements

Samples of E1010 bulk drug were exposed to various RH conditions at 25°C. The RH values for equilibration of the samples were controlled using saturated salt solutions with known RH values in desiccators.[32] The water content of each sample was analysed using a Model CA-05 Karl Fischer titrator (Mitsubishi Chemical Industries, Ltd, Tokyo, Japan). The samples (approximately 20 mg) were accurately weighed and quickly transferred to the titration vessel containing anhydrous methanol before titration. To evaluate the hygroscopicity for E1010 amorphous form, its vapour pressure isotherm was obtained using a dynamic vapour sorption (DVS) analyser (VTI Corporation, Hialeah, FL, USA). In this system, approximately 20 mg of the amorphous form was suspended in an isothermal chamber at 25°C, and the RH was regulated from 5% to 95% by adjusting the relative flow rates of dry (0% RH) and moist (100% RH) nitrogen. The weight of the sample was measured every 2 min with the microbalance. The weight stability criterion employed for the equilibrium was that the maximum weight change for each measurement was less than 0.2% (w/w).

Thermal analysis

The thermogravimetric-differential thermal analysis (TG-DTA) and the DSC were performed using a Rigaku Thermoflex TAS200 TG8101D (Rigaku, Tokyo, Japan), and a Rigaku Thermoflex TAS200 DSC8240D (Rigaku, Tokyo, Japan) or a DSC-60 (Shimadzu, Kyoto, Japan), respectively. The temperature axis and the cell constant in both thermal analyses were calibrated with indium. The solid samples (approximately 10 mg) were accurately weighed into open aluminum

pans, and then heated under a non-purge atmosphere from room temperature to 200°C at 2°C/min using an empty pan as reference.

Chemical stability studies

E1010 chemical stability in the solid state was evaluated at 60°C for two weeks. E1010 solid sample was weighed and then placed in a 10 ml screw-capped vial. Each stressed sample was dissolved in 20 mM phosphate buffer with 50 mM sodium perchlorate (pH 6.5) to assay E1010 content by high-performance liquid chromatography (HPLC). The HPLC analyses were performed with a Shimadzu LC10A system (Shimadzu, Kyoto, Japan) equipped with a 6.0 × 150 mm YMC-Pack ODS (YMC, Kyoto, Japan), which was maintained in a column oven at 30°C. Mobile phase A was 20 mM phosphate buffer with 50 mM sodium perchlorate (pH 6.5). Mobile phase B was 60% (v/v) acetonitrile and 40% (v/v) 20 mM phosphate buffer with 50 mM sodium perchlorate (pH 6.5). The gradient program was as follows: 0–5% B for 2 min, 5% B for 8 min, 5–30% B over 15 min, and 30–100% B over 5 min. The flow rate was 1.5 ml/min, and the detection wavelength was 297 nm.

Results and Discussion

Characterization of the E1010 bulk drug

A single crystal X-ray analysis for E1010 was carried out to solve the crystal structure using a plate crystal (approximate dimensions: 1.0 × 0.4 × 0.1 mm). The crystal data are summarized in Table 1. This single crystal was found to be isomorphous with E1010 bulk drug as confirmed by comparison of the theoretical XRD pattern obtained from the crystal structure analysis and the experimental XRD pattern. The Oak Ridge thermal ellipsoid plot (ORTEP) drawing depicted in Figure 1 shows that the asymmetric unit consisted of a molecule of E1010 free form, a chloride ion and three water molecules, and the crystalline form was a trihydrate. In the structure, the nitrogen atoms in two pyrrolidine rings were protonated and the carboxyl group on the carbapenem ring was dissociated. The crystal packing view (Figure 2) implied that the crystals were formed in two layers connected by hydrogen bonds. One layer consisted of E1010 free form molecules, while the other consisted of chloride ions and water molecules along the c-axis. These results elucidated that E1010 bulk drug exists as a crystalline trihydrate formed in two layers. The water content of the bulk drug was 11.4 ± 0.2% ($n = 6$), which was consistent with the theoretical value (10.8%) for the trihydrate crystal. Neither water content nor XRD pattern of the bulk drug changed in the humidity range from 11% to 93% RH at 25°C (data not shown). In contrast, E1010 amorphous form was very hygroscopic and exhibited deliquescence when exposed to moist conditions.

Table 1 Crystal data of E1010 trihydrate

Space group information	
Crystal system	Monoclinic
Space group	$P2_1$
Z value	2
Unit cell parameters	
a	9.565 (1) Å
b	10.990 (1) Å
c	12.431 (2) Å
β	109.336 (9)°
V	1233.1 (3) Å^3

Z value is molecular formula unit per crystal unit cell, a is the lattice parameter of a-axis, b is the lattice parameter of b-axis, c is the lattice parameter of c-axis, β is the angle between a-axis and c-axis, V is crystal volume per unit cell.

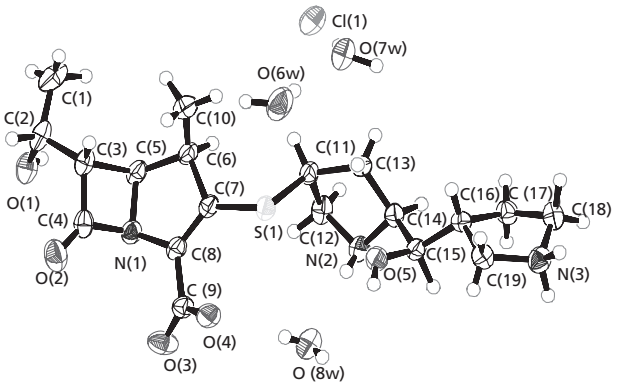

Figure 1 Oak Ridge thermal ellipsoid plot drawing for E1010 trihydrate crystal, showing 50% probability displacement ellipsoids.

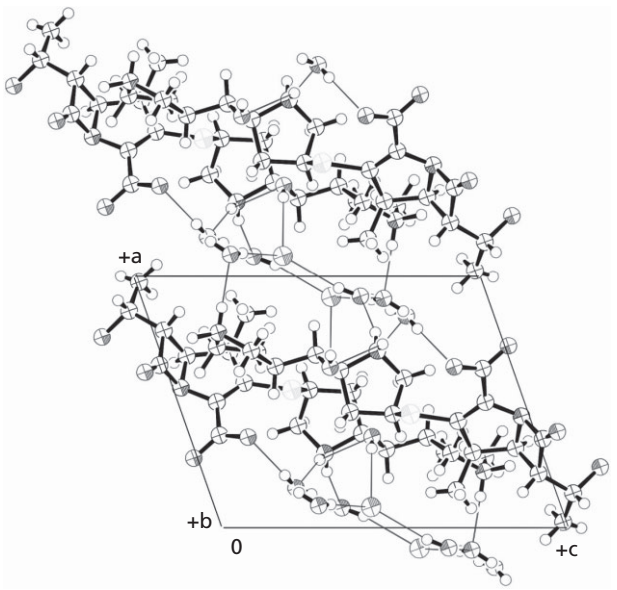

Figure 2 Three dimensional view of the supramolecular layer in E1010 bulk drug.

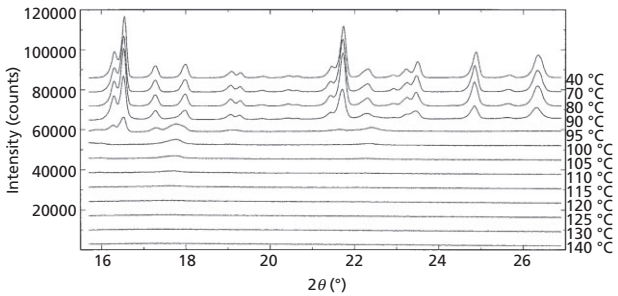

Figure 3 X-ray powder diffraction patterns of E1010 bulk drug at various temperatures.

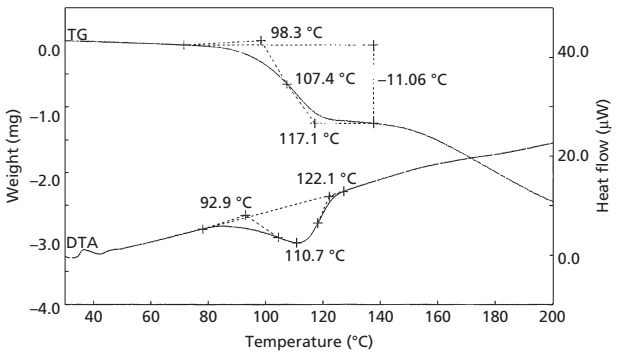

Figure 4 Thermogravimetric-differential thermal analysis thermogram of E1010 bulk drug at the heating rate of 2°C/min. TG, thermogravimetric; DTA, differential thermal analysis.

When the bulk drug was heated in a stepwise manner from 40°C to 140°C, the intensities of Bragg peaks decreased gradually, and then completely disappeared at or over 105°C (Figure 3). This phenomenon suggested a transformation of the crystalline trihydrate into the amorphous form rather than melting, because a solid state was still observed at or greater than 140°C by visual inspection. In the TG-DTA thermogram of the bulk drug, ~11% of weight loss was observed at the temperature range from 90°C to 140°C, where the DTA curve showed an endothermic peak (Figure 4). This endothermic peak implies dehydration behaviour of the bulk drug, because the weight loss was comparable with the water content of the crystalline trihydrate. The DSC thermogram of the bulk drug also showed an endothermic peak at around 100°C as an onset, while that of the amorphous form did not show any endothermic peaks under the same measurement conditions (Figure 5). These thermal analyses indicated that the endothermic peak came from dehydration of crystallization water in the bulk drug. The above variable temperature XRD measurements proved that amorphism of the bulk drug occurred up to 140°C. Consequently, loss of water in the crystalline trihydrate resulted in amorphism and thus the enthalpy change obtained by DSC was thought to be related to its crystallinity.

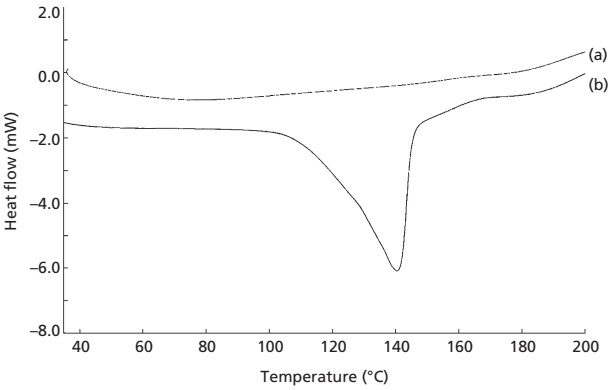

Figure 5 Differential scanning calorimetry thermograms of E1010 amorphous and crystalline forms in an open pan at the heating rate of 2°C/min. (a) E1010 amorphous form. (b) E1010 crystalline trihydrate.

Quantitative analysis of crystallinity

In numerous analytical techniques to assess crystallinity, XRD will be the most definitive method of detecting crystalline forms.[14–17] However, it is sometimes unsuitable for quantification analysis because diffraction intensity is affected by the dimensions of solid samples. Spectroscopic methods such as IR, Raman and NIR spectroscopy are useful for quantifying both amorphous and crystalline phases in solids, once appropriate spectral features of both phases have been identified.[14,25–27]When an amorphous material crystallizes with changes in humidity, DVS detects the crystallization response of the amorphous material, where extent of water sorption and desorption is related to the amorphous content of the sample.[18,19]

Quantification of crystallinity for E1010 bulk drug was performed using the values of enthalpy change observed in DSC thermograms. DSC is a fast and comparatively easy technique and requires smaller amounts of sample when compared with other methods. As described in Figure 5, no definite endothermic peak appeared in the amorphous form. In the TG-DTA thermogram of the amorphous form, the weight loss (~9.7%) due to dehydration of water of adhesion was not accompanied by any endothermic peaks (data not shown). Hence, these thermal analyses supported that the enthalpy change value represented a dehydration process of only crystallization water and was a practical index to determine the crystallinity of the bulk drug.

Since degree of crystallinity in a bulk drug can be defined as the percent crystalline content in the total sample, binary physical mixtures of the crystalline trihydrate and the amorphous form (amorphous content: 0–70% (w/w)) were used to make a calibration curve for the quantitative analysis. Specifically, the values of enthalpy change obtained by DSC experiments executed in triplicate were plotted against the percent ratio of the crystalline trihydrate in physical mixtures. As a result, an excellent linearity was observed between the

crystalline ratio (x-axis) and the enthalpy change (y-axis), though some errors introduced by mixing and sample segregation can be included in the relationship. The least squares linear regression gave a slope of 3.58, an intercept of −48.3, and a coefficient of correlation of 0.998. Good reproducibility was also confirmed because the %RSD values ($n = 3$) in the samples containing 0% and 5% of the amorphous form were 0.45% and 1.40%, respectively. Based on these results, this DSC method was found to quantify the degree of crystallinity of E1010 bulk drug accurately and to detect the difference of amorphous content among production batches at the level of a few percent. The reason for the negative intercept may have been that the measured values of enthalpy change for lower crystallinity samples were slightly underestimated due to factors such as baseline drift of the DSC traces. A mixing operation with the amorphous form also might have caused the decrease in the crystallinity of the crystalline trihydrate, and then impact the intercept of the calibration curve. However, such a defect in the evaluation for lower amounts of crystalline content in predominantly amorphous samples will not impact the quality control of the bulk drug where small amounts of the amorphous form have to be detected.

Relationship between crystallinity and chemical stability

The degree of crystallinity often influences the chemical stability of pharmaceutical bulk drugs and causes variability among production lots.[1–4] This is due to the content of amorphous material produced in manufacturing processes, e.g. recrystallization and milling. E1010 amorphous form was less stable than its crystalline forms under stressed conditions, as is the case in other carbapenem antibiotics, and thus some lots were found to be less stable than others.[5–7,31] Accordingly, the chemical stabilities in the solid state for nine lots with different values of enthalpy change were compared to determine the relationship with the degree of crystallinity. E1010 lots 1 to 6 were prepared by recrystallization from 2-propanol/water solvent systems (approximately 20–45% (v/v)). Lots 7 and 8 were prepared by milling of lot 2 with a planetary ball mill, P-5 (FRITSCH GmbH, Idar-Oberstein, Germany) for 5 min at rotation values of 5 and 9, respectively. Lot 9 was the physical mixture (95 : 5, w/w) of lot 1 and the amorphous form. As a result, E1010 content of the stressed samples, which were stored at 60°C for two weeks, became lower and lower as their enthalpy change decreased. When the residual ratio, i.e. the E1010 percentage against the initial content in the stressed samples, was plotted against the value of enthalpy change determined by DSC, a positive correlation with a coefficient of correlation of 0.956 as shown in Figure 6 was observed. The bulk lots with enthalpy changes of not less than 320 J/g showed no significant decrease in the residual ratio under the stressed condition. Therefore, it is suggested

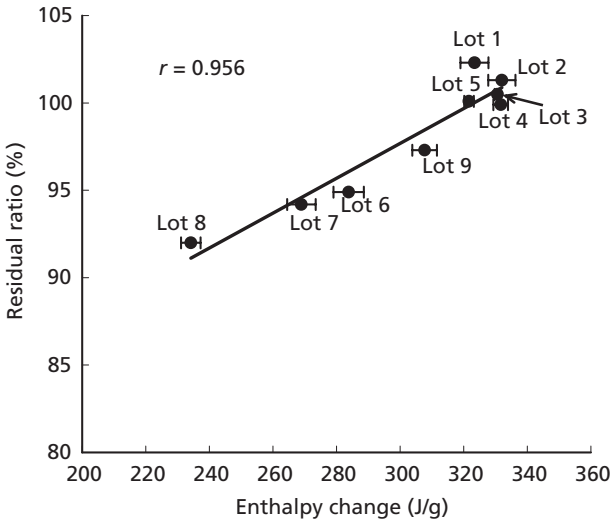

Figure 6 Relationship between enthalpy change and chemical stability in solid state of E1010 bulk batches. The error bars of the x-axis represent the standard deviation of enthalpy change ($n = 3$). *r*, a coefficient of correlation.

that the value of enthalpy change is a valuable surrogate marker to estimate the chemical stability of E1010 bulk drug and to distinguish batch-to-batch variability.

Conclusions

E1010 bulk drug was found to exist in a crystalline trihydrate. It was non-hygroscopic and did not show any crystal transformation under humid conditions. In thermal analyses, the bulk drug showed an endothermic peak due to dehydration with the disappearance of crystal lattices at around 100°C as an onset. The enthalpy change for this endothermic peak obtained by DSC correlated well with the degree of crystallinity and was applicable to the quantification of crystallinity of the bulk drug. This enthalpy change was found to be a useful index to estimate the chemical stability of the bulk drug, because it showed a positive relationship with the percent E1010 content of the stressed samples. DSC is a fast, accurate, and comparatively easy method to determine enthalpy change. Therefore, the use of DSC can facilitate the quality control of the bulk drug containing small amounts of the amorphous form.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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