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Thermal behavior and thermal decarboxylation of 10-hydroxycamptothecin in the solid state

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Abstract

In order to investigate the thermal-related properties and thermal stability of 10-hydroxycamptothecin (10-HCPT) in the solid state, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transform infrared (FT-IR) microspectroscopy were used. A novel combination of FT-IR microspectroscopy with thermal analyzer was applied simultaneously to monitor the dehydration and rehydration processes of the 10-HCPT sample. The thermal-induced decomposition of the 10-HCPT sample was also determined by using electrospray-ion trap mass spectrometry (ES-ITMS). The results indicated that the 10-HCPT sample used in this study was a monohydrate in structure, this form that can dehydrate to an anhydrate form if the temperature goes beyond 90 °C. The 10-HCPT anhydrate was first suggested to have two polymorphs, in which the form I might transform to form II when the 110 °C-preheated sample was cooled to 30 °C. The polymorphic transformation temperature was shown within 90–120 °C with 10.46 kcal/mol of enthalpy. The peak at 1723 cm^{-1} found in the IR spectrum of 10-HCPT monohydrate might correspond to the hydrogen-bonded C=O stretching vibration of lactone, which shifted to 1750 cm^{-1} assigned to a free C=O group of lactone after the destruction of hydrogen bonding via dehydration. This suggests that monohydrate seems to interact intramolecularly with 10-HCPT by hydrogen bonding. However, the rehydration process of the 10-HCPT anhydrate might cause it to return to being a monohydrate, depending on the storage condition. In addition, the thermal-induced decarboxylation of the solid-state 10-HCPT when the temperature is beyond 226 °C was proven by the appearance of a new IR peak at 1701 cm^{-1} and one major mass spectral peak at *m/z* 321. This unique IR spectral peak at 1701 cm^{-1} was due to the conjugated carbonyl group in the degraded product of 10-HCPT. The *m/z* 321 assigned to the decarboxylation of 10-HCPT was equal to the molecular weight loss of 44 from mass spectra; which was consistent with the weight loss of 11.9% (molecular weight of 43.3) from TGA curve of 10-HCPT anhydrate.

Keywords: 10-HCPT; Dehydration; Rehydration; Thermal behavior; Decarboxylation; DSC; TGA; FT-IR/DSC; Mass spectroscopy; Chemical stability

1. Introduction

10-Hydroxycamptothecin (10-HCPT) is one of the camptothecin (CPT) analogues with a powerful cytotoxic effect for cancer therapies [1–3]. 10-HCPT has a unique mechanism that allows it to inhibit the DNA topoisomerase I of tumors and at the same time, control the proliferation of cancer cells. When the aromatic A ring of the quinoline moiety in the CPT structure is hydroxylated at position C-10 to form a hydroxylated product (Fig. 1), it is more active and less toxic than CPT. In addition, the 20*S*-isomer of 10-HCPT is 10–100 times more able than the 20*R*-isomer to show anticancer activity [4,5].

The structure–activity relationship of CPT shows that the lactone ring is an essential pharmacophore in the ring E structure of CPT to have the antitumor activity, despite the fact that the lactone ring is pH-liable [6,7]. It has also been reported that higher chemical reactivity of α-hydroxylactone may induce a rapid equilibration between the lactone form and the ring-opened carboxylate form in physiological conditions [8,9]. Although both forms of 10-HCPT are effective against tumor growth, the lactone form is more effective than carboxylate form [10]. This implies that the process of keeping the lactone ring in the 10-HCPT structure is the key factor in controlling the stability and efficacy of 10-HCPT. Since oral solid dosage is for the most con-

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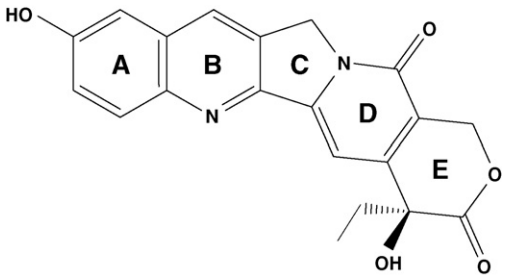


Fig. 1. Chemical structure of 10-hydroxycamptothecin (10-HCPT).

venient form of drug administration, many solid formulations of 10-HCPT have been developed to protect the lactone ring in the 10-HCPT structure from the rapid ring-opening process [11–13]. Thus, more attention should be paid to the basic properties of the solid-state 10-HCPT, including the thermal behavior and thermal stability in the solid state.

A number of studies have focused on the relationship between lactone-stability and structure–activity of 10-HCPT [3,14,15], but there is lack of study on the solid-state properties for the raw material of 10-HCPT. The intramolecular hydrogen bonding between the 20*S*-hydroxyl and the carbonyl of lactone has been found not only to activate the lactone but also to diminish the interaction with DNA topoisomerase [15]. Consequently, we find it quite interesting to study 10-HCPT in its solid state. In the present study, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transform infrared (FT-IR) microspectroscopy were applied to investigate the solid-state properties of 10-HCPT [16,17]. At the same time, in order to determine the correlation between the thermal response and the structural change in the 10-HCPT structure, a novel technique of FT-IR microspectroscopy equipped with a thermal analyzer was also used [18,19]. In addition, an electrospray-ion trap mass spectrometry (ES-ITMS) was used to examine the thermal-induced degradation of the 10-HCPT powder in the solid state.

2. Experimental

2.1. Materials

10-Hydroxycamptothecin (10-HCPT, pharmaceutical grade) was prepared by Huangshi Fy Pharmaceutical Co. Ltd. (Huang Shi, Hubei, China) as a powder. The KBr crystal was of analytical reagent grade and purchased from Jasco Parts Center (Jasco Co., Tokyo, Japan).

2.2. Thermal analysis of 10-HCPT

A certain amount of the 10-HCPT powder was examined by using differential scanning calorimetry (DSC; DSC-910, TA Instruments Inc., New Castle, DE, USA) at a heating rate of 3 °C/min with an open pan system from 30 to 300 °C, both in a stream of N_2 gas and without N_2 gas. The DSC cell was calibrated with indium. In addition, several samples were respectively preheated to a prescribed temperature and cooled to 30 °C, and then reheated with the DSC system. The enthalpy was performed in triplicate to provide mean values and standard deviations (S.D.). Thermogravimetric analysis (TGA; TGA-951, TA Instruments Inc., New Castle, DE, USA) was also performed at the same heating rate without N_2 gas to measure the weight loss of the sample. The typical amount of sample used on DSC and TGA studies were about 10 mg.

2.3. FT-IR microspectroscopic determination

A trace of the 10-HCPT powder was either smeared on only one piece of KBr pellet without further compression (the smeared method) or sealed into two pieces of KBr pellets with a compression pressure of 200 kg/cm^2 for 15 s (also known as the compressed method). After each treatment, all the KBr samples were determined by using FT-IR microspectroscopy (Micro FTIR-200, Jasco, Japan) with a mercury cadmium telluride (MCT) detector by a transmission mode. Several of the 200 °C-preheated 10-HCPT samples that were stored at different relative humidity (RH) conditions were also investigated.

2.4. Thermal FT-IR microspectroscopic study

The KBr samples prepared by either the smeared or compressed method mentioned previously were placed onto a micro hot stage (DSC microscopy cell, FP 84, Mettler, Greifensee, Switzerland). The DSC microscopy cell was then directly placed in an FT-IR microspectroscopy in transmission mode. The temperature of the DSC microscopy cell was monitored with a central processor (FP 80HT, Mettler, Switzerland). The heating rate of the DSC assembly was maintained at 3 °C/min in the room temperature (25 °C, 82% RH). The KBr sample was previously equilibrated to the starting temperature (30 °C) and then heated from 30 to 300 °C. The thermal-responsive IR spectra were then recorded [20,21].

2.5. Rehydration process

(1) The different 200 °C-preheated 10-HCPT samples were stored at 25 °C under 27, 39 or 69% RH for 4 h, and then determined their IR spectra.
(2) The rehydration process of each 200 °C-preheated 10-HCPT sample from 200 to 30 °C was investigated by a non-isothermal FT-IR measurement in the 25 °C, 82% RH condition.
(3) The above sample was further studied isothermally in a 25 °C, 56% RH condition with an isothermal FT-IR microscopic system by a time-scanned program for 4 h, and their IR spectra was continuously recorded.

2.6. Mass spectroscopy

The raw material and the 265 °C-preheated 10-HCPT samples were dissolved in methanol that was acidified with 0.1% acetic acid and then centrifuged. The supernatant was then sampled for analysis. Finnigan LCQ ion-trap mass spectrometer (Finnigan Corp., CA, USA) equipped with a Finnigan electrospray source was used in the process [22]. The flow rate of the

syring pump was 20 μl/min. For ES-ITMS, the capillary voltage was 26.0 V and the stainless steel capillary temperature was set to 180 °C. The pressure of the sheath gas was 35 psi and the spray voltage of ES-ITMS system was operated at 4.5 kV in the positive mode. Data acquisition was performed in the full scan mode from *m/z* 100 to 500 for ITMS with an injection time of 200 ms and 2 μ scans.

3. Results and discussion

3.1. Thermal analysis of the 10-HCPT powder

The DSC thermograms of the 10-HCPT powder after the different treatments are shown in Fig. 2. The TGA curve was obtained from the non-purging N_2 gas condition. Obviously, three steps of weight loss were found from the TGA curve for the 10-HCPT powder: 1.0% when the temperature was within 30–75 °C, 4.7% when the temperature was within 90–120 °C and 11.9% when the temperature was within 220–280 °C. The first weight loss of 1.0% within 30–75 °C was the desorption of previously adsorbed water [20,21]. The second weight loss of 4.7% within 90–120 °C might have been caused by the dehydration of water from the sample. After all, the 4.7% weight loss was almost equal to the loss of the 1 mol of water from the 10-HCPT powder [23]. Thus, the 10-HCPT powder used in the study may possibly be defined as a 10-HCPT monohydrate at first (molecular weight 382.4), but it gradually became a 10-HCPT anhydrate when the heating temperature went beyond 90 °C. Moreover, an endothermic peak at 110 °C with 11.99 ± 0.21 kcal/mol of enthalpy and two exothermic peaks at 223 and 250 °C were observed in the DSC thermogram under non-purging N_2 gas condition, as shown in Fig. 2a. The endothermic peak at 110 °C seemed to be consistent with the dehydration process in the TGA curve. The final weight loss of 11.9% within 150–280 °C might be due to the oxidation/degradation process of the sample, which was related to the exothermic peaks in the DSC curve [23–25]. Furthermore, an endothermic peak at 100 °C with 11.98 ± 0.18 kcal/mol of enthalpy and three exothermic peaks near 226, 254 and 276 °C were observed in the DSC thermogram under a continuously purging N_2 gas condition (Fig. 2b). The appearance of endothermic and exothermic peaks in the DSC thermogram under purging N_2 gas was clearly different from that of the DSC thermogram without purging N_2 gas; thus suggesting the importance of inert N_2 gas in thermoanalysis.

In order to verify each peak found in the DSC thermogram, the 10-HCPT monohydrate was previously heated from 30 to 110 °C or from 30 to 240 °C. Then it was cooled to 30 °C and reheated again by the DSC system under inert N_2 gas conditions. When the 110 °C-preheated sample was reheated, its DSC thermogram evidenced an endothermic peak at 96 °C with 10.46 ± 0.14 kcal/mol of enthalpy and another three exothermic peaks at 226, 254 and 276 °C (Fig. 2c), which was similar to the data of Fig. 2b. If the endothermic peak was related to the dehydration of water from sample, it should disappear from the DSC thermogram after the reheating process since the DSC system was maintained under dry N_2 gas conditions. The endothermic peak at 96 °C for the 110 °C-prereheated 10-HCPT sample after reheating still existed (Fig. 2c). However, it was only different on its peak position (100 versus 96 °C) and its enthalpy (11.98 ± 0.18 versus 10.46 ± 0.14 kcal/mol). Thus, the appearance of this endothermic peak might be considered as a polymorphic transformation for the 10-HCPT anhydrate. It is reasonable to deduce that the 10-HCPT monohydrate was first dehydrated to an anhydrate form of 10-HCPT at 90–120 °C, but this 10-HCPT anhydrate (defined as form I) transformed to another polymorph (defined as form II) when the heated sample was cooled to 30 °C. This suggests that the10-HCPT anhydrate may possess two polymorphs. However, the detailed preparation and identification of these polymorphs will be examined in the future by using X-ray powder diffraction, thermal and spec-

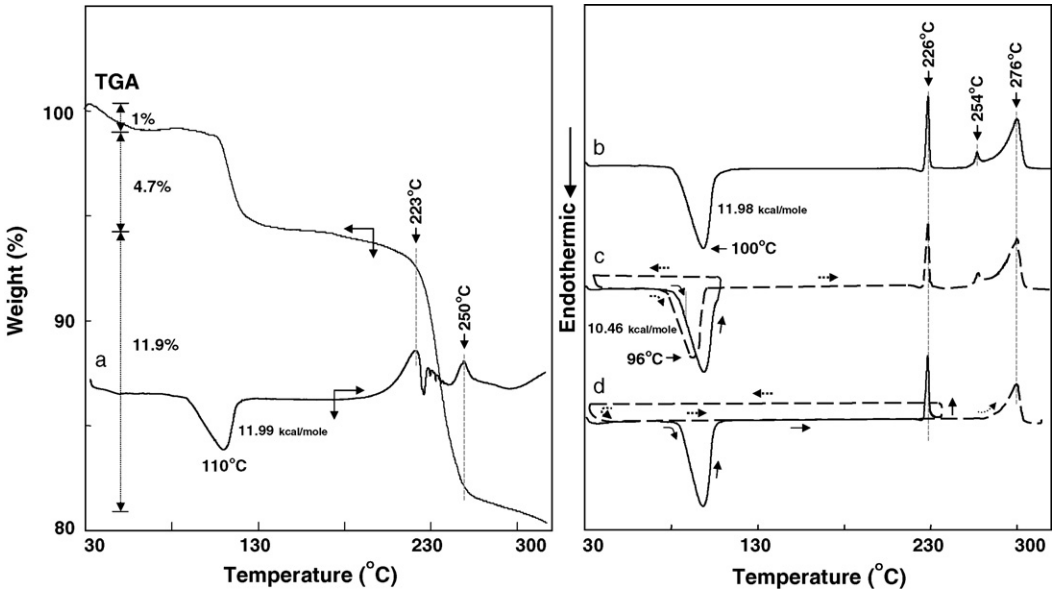


Fig. 2. TGA curve and DSC thermograms of 10-HCPT sample without (a) or with (b–d) purging N_2 gas. Key: (b) heated from 30 to 300 °C, (c) preheated to 110 °C (solid line) and then reheated (dash line), (d) preheated to 240 °C (solid line) and then reheated (dash line).

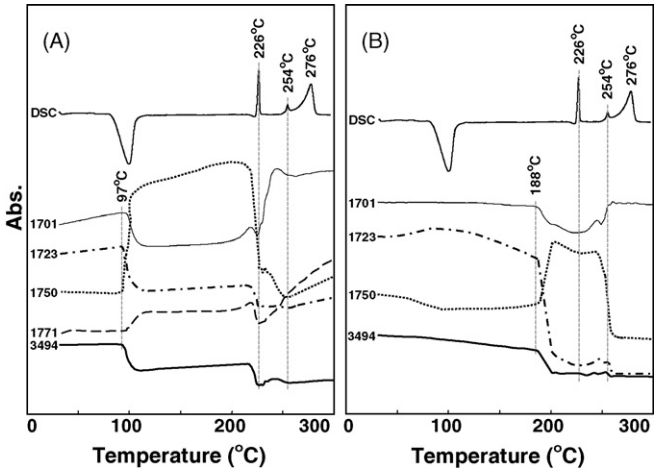


Fig. 3. DSC thermogram and thermal-dependent changes in the specified FT-IR spectra of 10-HCPT monohydrate prepared by smeared (A) or compressed (B) method.

troscopic techniques. When the 240 °C-preheated sample was reheated, the endothermic peak that was near 96 °C and two exothermic peaks at 226 and 254 °C were all absent from the DSC thermogram (Fig. 2d). The disappearance of these thermal peaks might be due to the thermal-related degradation of the 10-HCPT powder, which can be verified by the following ES-ITMS determination.

3.2. Thermal-dependent FT-IR spectral changes for the 10-HCPT monohydrate

Three-dimensional plots of the FT-IR spectra of the 10-HCPT monohydrate prepared by different methods as a function of temperature are obtained by determining with FT-IR microspectroscopy equipped with a micro hot stage. The FT-IR spectra of the 10-HCPT monohydrate prepared by both the smear and compressed methods at 30 °C are similar, implying that the method of compression has little effect. Several characteristic IR absorption bands and their assignments are shown as follows: 3494 and 3346 cm^{-1} (the O–H stretching mode), 3100–2750 cm^{-1} (the C–H stretching vibration), 1723 cm^{-1} (C=O stretching vibration of lactone), 1653 cm^{-1} (the C=O stretching vibration of amide), 1593 and 1502 (1503) cm^{-1} (the aromatic ring mode),

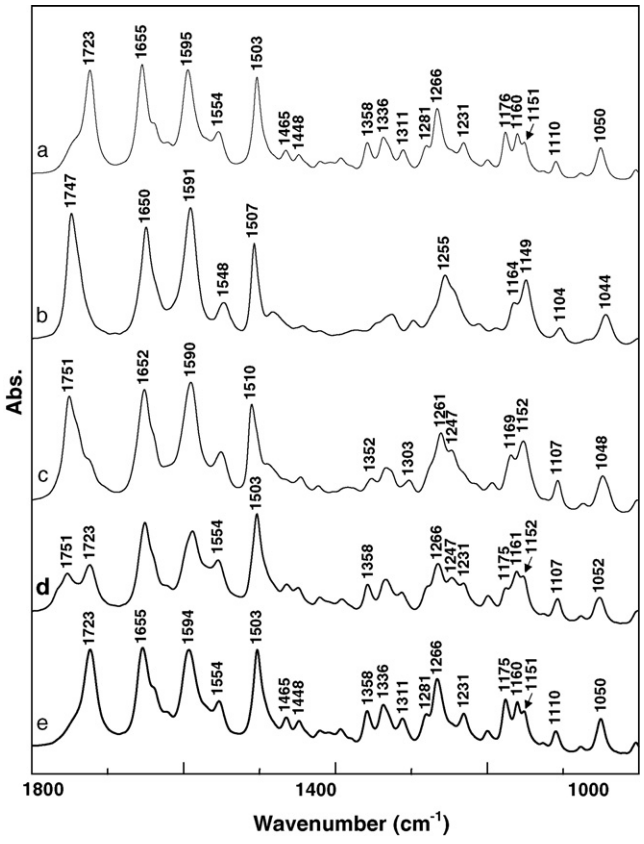
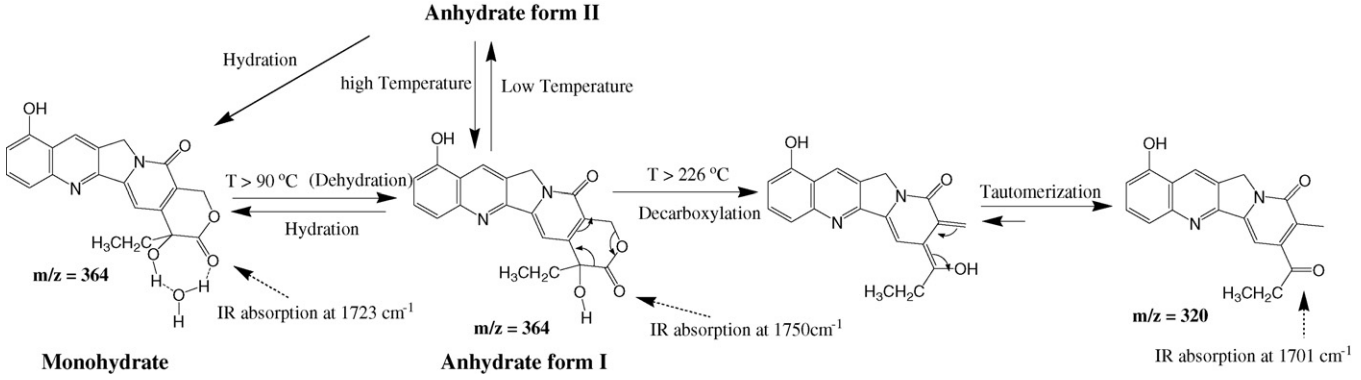


Fig. 4. FT-IR spectra of different 10-HCPT samples stored at various storage conditions. Key: (a) 10-HCPT monohydrate before preheating, (b) 10-HCPT monohydrate after heating to 200 °C, and 200 °C-preheated sample stored at (c) 27% RH condition for 4 h, (d) 39% RH condition for 4 h, and (e) 69% RH condition for 4 h.

1266 and 1051 cm^{-1} (the C–O and/or C–N stretching mode [26]. By increasing the temperature, however, two dramatic changes near 97 and 226 °C for the 10-HCPT monohydrate prepared by smeared method were noted from the contour profile of FT-IR spectra. The peak at 1723 cm^{-1} that was originally caused by the hydrogen-bonded C=O in lactone structure disappeared from 97 °C, but a new peak at 1750 cm^{-1} assigned to free carbonyl group of lactone gradually appeared when the temperature went beyond 97 °C and disappeared again at 226 °C (Scheme 1). In addition, two additional new peaks, at 1771



Scheme 1. Possible pathway for thermal-dependent dehydration/rehydration, polymorphic transformation and degradation of 10-HCPT sample.

and 1701 cm^{-1}, were gradually observed when the temperature exceeded 226 °C for the 10-HCPT sample. This might be due to the functional groups of the degraded products (evidenced by Fig. 2d). Compared to the 10-HCPT monohydrate that was prepared by the smeared method, the 10-HCPT monohydrate that was prepared by the compressed method showed clear changes at 188 and 226 °C, specifically in the contour profile of FT-IR spectra. The peak at 1723 cm^{-1} disappeared when the temperature went beyond 188 °C, and the peak at 1750 cm^{-1} was evident at from 188 °C but disappeared after 226 °C. There was only one peak at 1701 cm^{-1} that was present when the temperature reached 226 °C.

The changes in peak intensity for the representative IR bands of the 10-HCPT monohydrate with the increase of temperature are shown in Fig. 3. The peak intensity was obtained from the peak height via baseline correction. Because the atoms or molecules of 10-HCPT occupied the fixed site with very little conformational freedom in the solid state at lower temperature, the IR bands maintained their peak frequency and intensity consistently until thermal response. For the smeared sample, it is evident that the peak intensities at 3494 and 1723 cm^{-1} dropped sharply when the temperature reached 97 °C (Fig. 3A). At the same time, another IR peak at 1750 cm^{-1} quickly appeared at 97 °C after the dehydration of water and also strengthened its peak intensity until 226 °C (Scheme 1). The changes of these specific IR peak intensities at 97 °C for the solid-state 10-HCPT powder might be caused by the dehydration from monohydrate to anhydrate, which was confirmed by the temperature of dehydration that appeared in the DSC and TGA curves (Fig. 2). Beyond 226 °C, two new peaks at 1701 and 1771 cm^{-1} for the sample that was prepared by the smeared method were also found, which can be attributed to the functional groups of the degraded products. In contrast, the sample prepared by the compressed method induced an appearance of 1750 cm^{-1} peak at 188 °C rather than at 97 °C (Fig. 3B). Two peaks at 3494 and 1723 cm^{-1} constantly maintained their peak positions and intensities until 188 °C, when their peak intensities reduced. In addition, the temperature of dehydration for the 10-HCPT monohydrate prepared by the compressed method was delayed from 97 to 188 °C, as compared with that of the smear method. This delaying phenomenon during the dehydration process might be due to the sealing of the 10-HCPT monohydrate within the KBr pellets, which hinders the movement of water in the tight compact.

3.3. Rehydration of 10-HCPT anhydrate

Fig. 4 displays the FT-IR spectra of different 200 °C-preheated 10-HCPT samples stored at various RH conditions for 4 h. Here, the KBr sample was prepared by the compressed

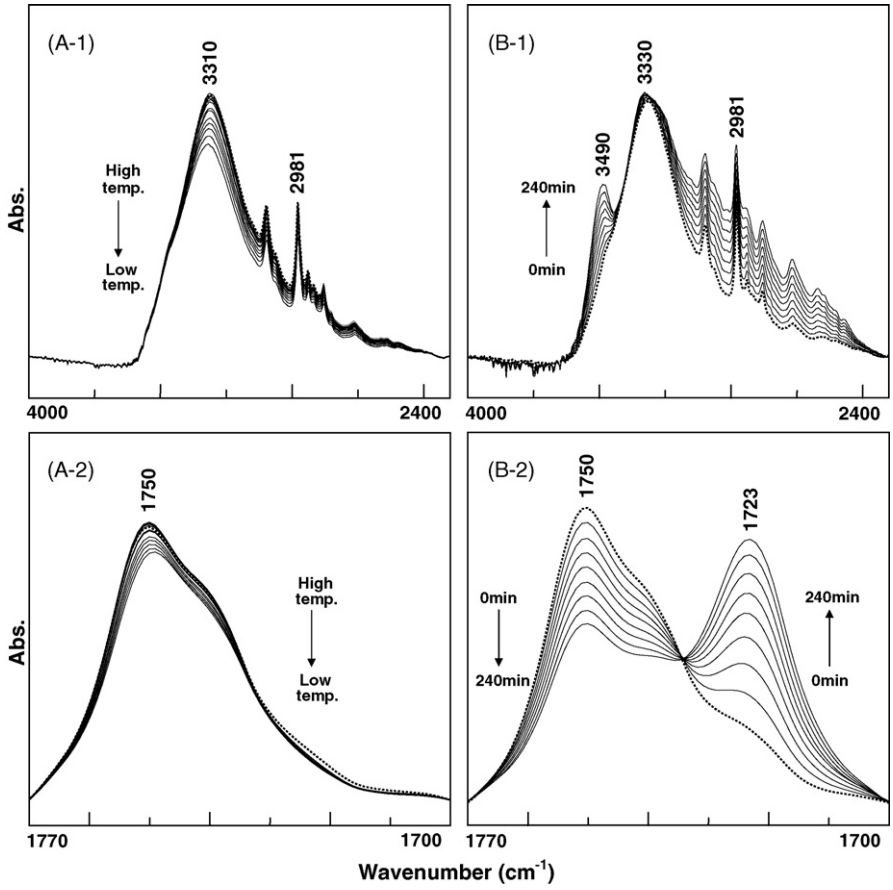


Fig. 5. Three-dimensional FT-IR spectral changes of 200 °C-preheated 10-HCPT samples prepared by compressed method in the cooling process (A-1 and A-2) and its isothermal three-dimensional FT-IR spectra at 25 °C, 56% RH condition for 4 h (B-1 and B-2).

method. When the 200 °C-preheated sample was stored at a 27% RH condition, its IR spectrum (Fig. 4c) was noticeably different from the IR spectrum of the raw material (Fig. 4a), but similar to the IR spectrum of the anhydrous sample (Fig. 4b). When the 200 °C-preheated sample was stored at a 69% RH condition, however, the anhydrate of 10-HCPT completely returned to a monohydrate (Fig. 4e). On the other hand, once the 200 °C-preheated sample was stored at a 39% RH condition for 4 h, a mixture of both monohydrate and anhydrate of 10-HCPT was obtained, as shown in Fig. 4d. It should be noted that in both cases, the IR spectral frequency of the C=O stretching vibration of six-ring lactone was always located at 1740 cm^{-1} [27]. However, when the C=O stretching vibration of lactone in the 10-HCPT structure appeared at 1723 cm^{-1} it was reasonable to suppose that an extra intermolecular interaction, such as hydrogen bonding, existed in the 10-HCPT structure. The monohydrate seems to interact intramolecularly and/or intermolecularly with the 10-HCPT in the 10-HCPT monohydrate structure by hydrogen bonding, as shown in Scheme 1. Once the sample became dehydrated, the location of the IR peak for the non-hydrogen-bonded C=O stretching vibration of lactone appeared at high wavenumber positions of either 1747 or 1751 cm^{-1}.

In order to further confirm the detailed rehydration process of the 10-HCPT anhydrate, the 200 °C-preheated 10-HCPT sample was investigated by a non-isothermal FT-IR measurement in the room temperature condition (25 °C, 82% RH). The 10-HCPT monohydrate sample prepared by the compressed method was preheated to 200 °C isothermally for 10 min, then cooled from 200 to 30 °C; the changes in three-dimensional FT-IR spectra from higher temperature to lower temperature are shown in Fig. 5A. In the cooling course, which lasted 60 min, the IR spectral peaks at 3490 and 1723 cm^{-1} were absent but the peak at 1750 cm^{-1} still existed. This might be due to the fact that the anhydrous 10-HCPT still existed during this cooling period. Since the 10-HCPT sample was embedded and tightly compacted within two pieces of KBr pellets, the rehydration process of 10-HCPT sample failed to occur. However, once the cooled sample was further studied isothermally in a 56% RH condition with an isothermal FT-IR microscopic system with a time-scanned program for 4 h at 25 °C, the peak at 3494 cm^{-1} gradually increased its peak intensity with time. Meanwhile, the peak intensity at 1750 cm^{-1} that was assigned to the free C=O stretching vibration of the anhydrate state slowly decreased, though it still remained (Fig. 5B). However, at 1723 cm^{-1}, the peak intensity that was assigned to the hydrogen-bonded C=O stretching vibration of the monohydrate state was significantly enhanced, implying that a mixture of monohydrate and anhydrate of 10-HCPT was found in this storage condition. This revealed that under a 25 °C, 56% RH condition, the 200 °C-preheated 10-HCPT sample prepared by the compressed method will not completely recover to a 10-HCPTmonohydrate.

3.4. Thermal-induced degradation of the 10-HCPT samples

The intact 10-HCPT, 265 °C-preheated 10-HCPT samples were evaluated by using electrospray-ion trap mass spectrometry (ES-ITMS) to determine the molecular weights of the degraded products, as shown in Fig. 6. Only one mass spectral peak, at 365, was found, coming from the $[C_{20}H_{16}N_2O_5 + H]^+$ peak for the raw material of 10-HCPT (molecular weight 364). However, one major mass spectral peak at 321 was observed alone for the 265 °C-preheated 10-HCPT sample. The peak at 321 was caused by the decarboxylated 10-HCPT sample since it appeared at a lower molecular weight than the parent drug, by 44 mass units. The molecular weight loss from mass spectra was consistent with the weight loss of 11.9% (equal to molecular weight of 43.3) from the TGA curve of the 10-HCPT anhydrate. The thermal-induced decarboxylation of lactone is well known [27] and as a result, it is therefore reasonable to assume that the thermal-induced decarboxylation from lactone ring in the 10-HCPT structure might occur at high temperatures. Thus, the proposed Scheme 1 is a possible reaction pathway for the thermally induced degradation of the 10-HCPT anhydrate. Since a unique IR peak at 1701 cm^{-1} was found in Fig. 3 when the 10-HCPT sample was heated beyond 226 °C, we propose that a carbonyl group might exist in the decarboxylated 10-HCPT sample. However, the formation of tautomerization of the enolic group in the degraded 10-HCPT product is assumed.

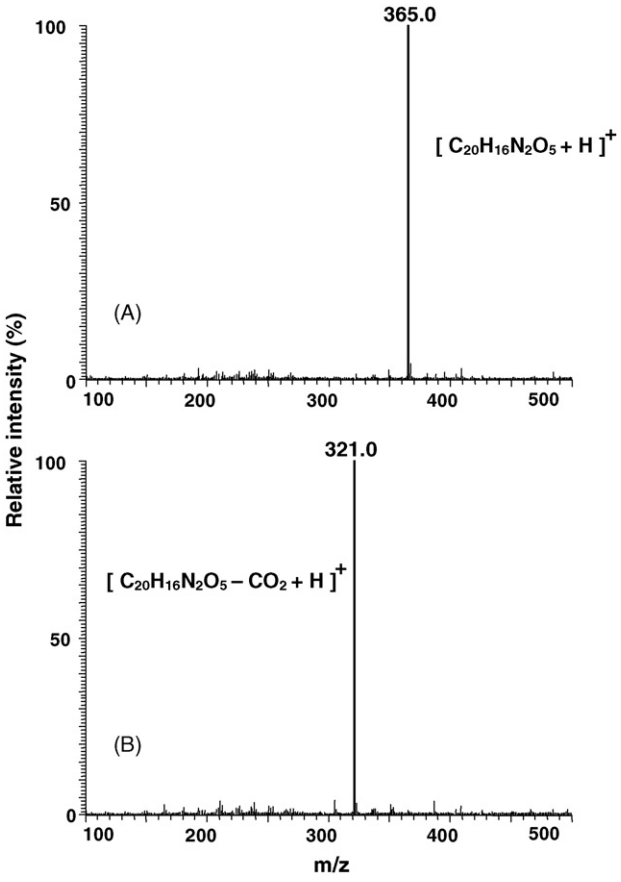


Fig. 6. Mass spectra for native 10-HCPT (A) and 265 °C-preheated (B) 10-HCPT samples.

4. Conclusions

The 10-HCPT sample used in this study was a monohydrate. This 10-HCPT monohydrate could thermally be dehydrated

to form an anhydrate near 100 °C. Moreover, the 10-HCPT anhydrate had two polymorphs, in which the enthalpy of this polymorphic transformation was 10.46 kcal/mol. The 10-HCPT anhydrate could be rehydrated to a monohydrate at a high RH condition. At a high temperature, the solid-state decarboxylation of the lactone ring was the main thermally induced degradation mechanism for the 10-HCPT anhydrate.

This paper is just a starting point towards a deeper investigation of the 10-HCPT sample. The physicochemical characterizations of the solid-state 10-HCPT such as the preparation and properties of different crystal forms, physical stability, etc., will be studied by X-ray diffraction, thermal and spectral determinations in the future.

Acknowledgements

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