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Short communication

Evaluation of hydrate formation of a pharmaceutical solid by using diffuse reflectance infrared Fourier-transform spectroscopy

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Abstract

Ampicilline and nitrofurantoin, in both anhydrous and hydrate forms, were characterized by powder X-ray diffractometry (PXRD), thermogravimetric and differential thermal analyses (TG/DTA) and diffuse reflectance FT-IR spectroscopy (DRIFTS). Of all the analytical tools applied, only DRIFTS was able to indicate the formation of hydrogen bonds between the molecules of the anhydrous drug substance and crystalline water uptaken from atmospheric moisture as evidenced by the significant absorption at 3500–3700 cm^{-1} corresponding to crystal water. These results suggested that DRIFTS could provide information on hydration without a standard sample and accurately evaluate the physical stability focusing on the qualification of slight hydration in the early stages of pharmaceutical development. In addition, DRIFTS was applied to the besylate salt of pharmaceutical compound A to identify any possible hydration. This salt had the stable form BSA-I, metastable form BSA-II and hydrate form BSA-III. DRIFTS was able to show the hydration of BSA-II even when stored in a capped bottle, eventually leading to the transformation into BSA-III, which was not detected by PXRD. These findings verify the usefulness of DRIFTS for the solid-state characterization of pharmaceutical substances, especially the monitoring of gradual hydration.

Keywords: Diffuse reflectance; Infrared spectroscopy; Hydrate; Ampicilline; Nitrofurantoin

1. Introduction

Solid form selection based on physicochemical properties such as solubility [1], stability [2,3] and manufacturability [4] was recently conducted in the early stages of pharmaceutical development in order to avoid excess investment in development and patent litigation [5]. In particular, both physical and chemical stability were identified as being essential in assuring the potency of compounds for *in vivo* experiments. In the early stages of development, metastable forms are often used for *in vivo* experiments. The risk does exist that they could transform into a stable form including hydrate resulting in a decrease in solubility. To realize the evaluation of physical stability in the early stages, an effective methodology providing multiple pieces of information, such as the evaluation of hydration without standard hydrate, with small amount of bulk is required.

Generally, physical stability is evaluated using powder X-ray diffractometry (PXRD), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). These analyses are all well established. However, they require standard samples such as hydrate to identify the transformation. As one of the alternative techniques, infrared (IR) spectroscopy was also used for solid-state characterization since it provided insight into the nature of polymorphism at the molecular level [6]. In particular, diffuse reflectance FT-IR spectroscopy (DRIFTS) emerged as the most important technique, and several stud-

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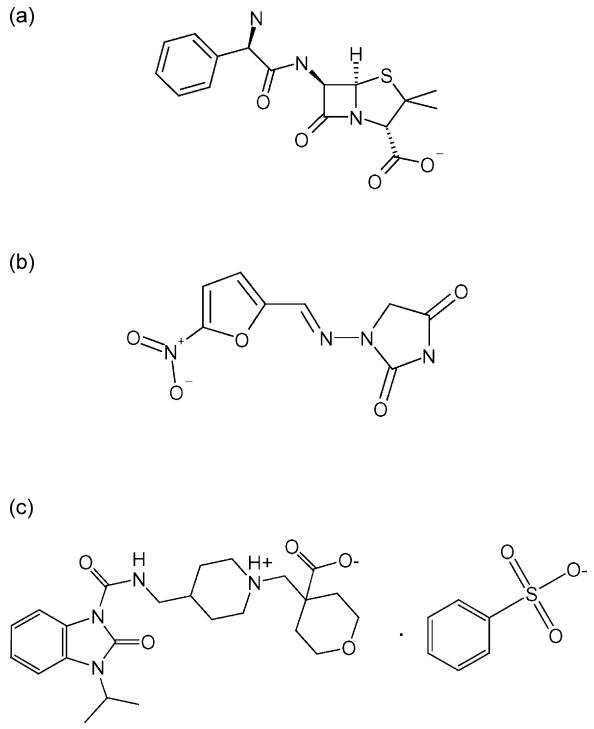

Fig. 1. Chemical structures of ampicilline, nitrofurantoin and compound A.

ies on characterization using DRIFTS have been published [7–9].

In this study, we prepared ampicilline and nitrofurantoin in their hydrate forms (Fig. 1), characterized them using PXRD, TG/DTA and DRIFTS, and discussed the application tool for physical stability in the early stage. In addition, DRIFTS was used to evaluate the qualification of hydration of besylate salt of pharmaceutical compound A (Fig. 1).

2. Materials and methods

2.1. Materials

Anhydrous ampicilline and nitrofurantoin were purchased from Wako Pure Chemical Industries (Osaka, Japan). 4-{[4-({[(3-Isopropyl-2-oxo-2,3-dihydro-1*H*-benzimidazol-1-yl)carbonyl]amino}methyl)piperidin-1-yl]methyl}tetrahydro-2*H*-pyran-4-carboxylic acid (compound A) was synthesized at Nagoya Laboratories, Pfizer Japan Inc. Trihydrate form of ampicilline [10], monohydrate form II of nitrofurantoin [11,12], and monohydrate of compound A [13] were obtained by recrystallizing saturated aqueous solutions of the drug while stirring at room temperature for a week. They were then filtrated and dried in a nitrogen atmosphere.

2.2. Powder X-ray diffractometry

Powder X-ray diffraction (PXRD) patterns were collected using a RINT-TTR (Rigaku, Tokyo, Japan) with Cu Kα radiation generated at 300 mA and 50 kV. Samples were placed on aluminum rotation-plates and rotated at 60 rpm at room temperature. Data was collected from 3° to 35° (2θ) at a step size of 0.02° and scanning speed of 4°/min.

2.3. Thermal analysis

TG/DTA was performed using a TG/DTA 6200 system (Seiko Instruments, Chiba, Japan). The TGA and DTA thermograms were obtained in an aluminum open-pan system using a sample weight of ca. 3 mg and a heating rate of 5 °C/min in a nitrogen flow.

2.4. Diffuse reflectance infrared Fourier-transform spectroscopy

The FT-IR spectra were recorded on a FTIR IRPrestige-21 (Shimadzu Corporation, Kyoto, Japan) with a diffuse reflectance accessory (DRS-8000, Shimadzu Corporation). The samples were diluted with KBr to give 5% (w/w) mixture for compound A. A total of 40 scans were collected on each sample with 4 cm^{-1} resolution. The spectra obtained were transformed to the Kubelka-Munk function for standardization.

3. Results and discussion

3.1. Solid-state characterization of ampicilline and nitrofurantoin anhydrous and hydrate

The powder X-ray diffraction (PXRD) patterns of ampicilline and nitrofurantoin in their hydrate forms agreed well with the data reported [10–12,14] and were identified as trihydrate of ampicilline and monohydrate of nitrofurantoin. Thermograms of thermogravimetry and differential thermal analyses (TG/DTA) also indicated that trihydrate of ampicilline and monohydrate of nitrofurantoin were obtained.

Significant differences were observed in the diffuse reflectance FT-IR spectral (DRIFTS) patterns between the anhydrous and hydrate forms of ampicilline (Fig. 2). The hydrate form showed a broad band around 3500 cm^{-1} attributed to

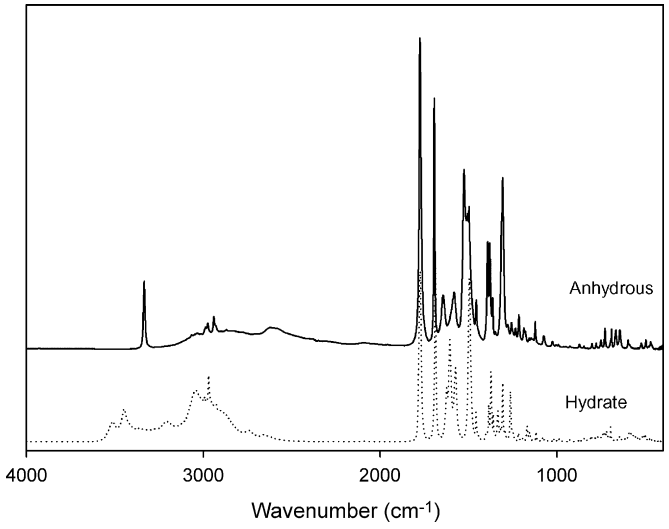

Fig. 2. DRIFT spectra of ampicilline anhydrous (—) and trihydrate (···).

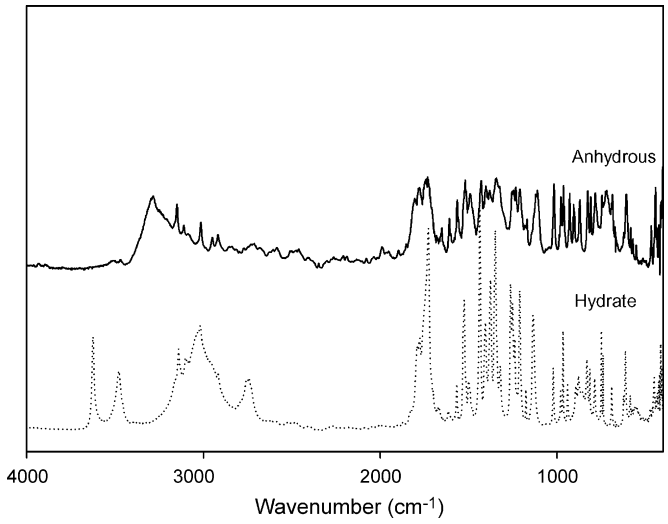

Fig. 3. DRIFT spectra of nitrofurantoin anhydrous (—) and monohydrate (· · ·).

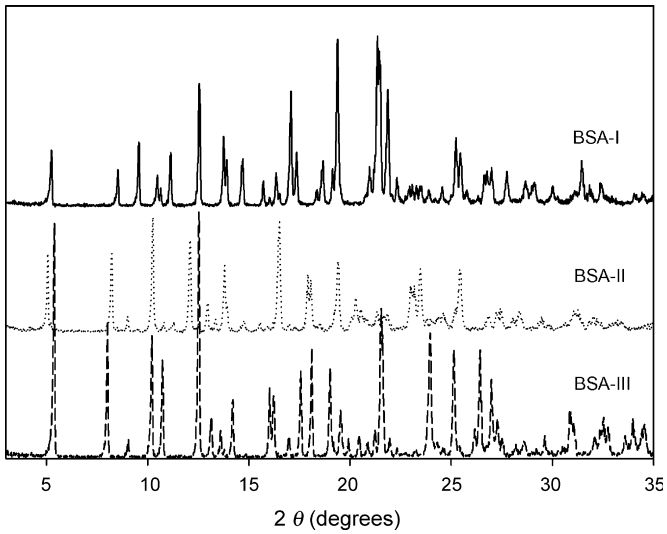

Fig. 4. PXRD patterns of BSA-I (—), BSA-II (· · ·) and BSA-III (- - -) stored at ambient condition.

hydrogen bonding between water and ampicilline molecules, whereas the anhydrous form showed no band in this region. The FT-IR spectral patterns of the anhydrous and hydrate forms of nitrofurantoin also exhibited significant differences (Fig. 3). The hydrate form showed sharp peaks around 3500 cm^{-1} attributed to hydrogen bonding between water and nitrofurantoin molecules, whereas the anhydrous form showed no peak in this region. These results suggested that in addition to the crystalline form, DRIFTS can also provide information on hydrogen bonding between water and pharmaceutical molecules caused by hydration.

In pharmaceutical development, physical stability focusing on hydration has generally been evaluated by both PXRD and thermal analyses such as TG/DTA. PXRD is able to provide detailed analysis for the transformation of crystalline forms. However, it failed to distinguish between transformation to the stable anhydrous form and hydration without standard samples of hydrate. On the contrary, TG/DTA might distinguish a difference from a loss of weight, taken together with the results of PXRD. In this study, we have demonstrated that DRIFTS can distinguish between transformation to the stable form and hydration based on information gathered as a result of hydrogen bonding, and be a useful application for the detection of the hydrate form.

3.2. Evaluation of hydrate formation of drug candidate

Pharmaceutical compound A had two anhydrous forms, BSA-I and BSA-II, and a hydrate form, BSA-III all of which are besylate salts [13]. We have reported that the stable form BSA-I was quite stable at 69%RH for at least 30 days without transformation. However, the hygroscopicity of the metastable form BSA-II was not discussed. In this study, PXRD and DRIFTS analyses of BSA-II stored in a capped bottle were conducted and compared with those of BSA-I and BSA-III. The PXRD patterns suggested that these crystals were quite different and very pure (Fig. 4). DRIFTS of the sample stored in a capped bottle, which was identified as BSA-II by PXRD, showed very

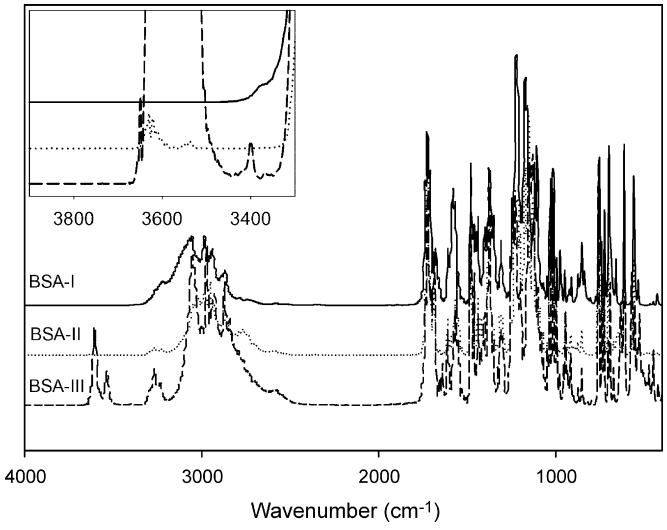

Fig. 5. DRIFTS spectra of BSA-I (—), BSA-II (· · ·) and BSA-III (- - -) stored at ambient condition.

weak bands around 3600 cm^{-1}, possibly attributed to hydrogen bonding between water and the besylate salt of compound A molecules, which was shown in hydrate, BSA-III. BSA-II stored in a capped bottle took up water from the atmospheric moisture during storage and transformed slightly to BSA-III, which was not detected by PXRD (Fig. 5). These results suggested that DRIFTS was indeed able to detect the slight hydration of BSA-II, and possibly the transformation to BSA-III, which was not detected by PXRD. In fact, BSA-II had completely transformed into BSA-III in the 2 years following this evaluation. Based on these findings, DRIFTS appears be a sensitive tool for the detection of hydration.

4. Conclusion

We have provided characterizations of the hydrate forms of ampicilline and nitrofurantoin by PXRD, TG/DTA and DRIFTS

and demonstrated that DRIFTS is a useful tool for physical stability studies focusing on the qualification of hydration in the early stages of pharmaceutical development. We have also demonstrated the application of DRIFTS for the evaluation of hydration of besylate salt of pharmaceutical compound A, and shown that DRIFTS is a sensitive tool for the detection of hydration.

Physical stability studies with small amounts of bulk are required in the early stages of pharmaceutical development. In some cases, the physical stability of the metastable form, which has the potential to transform into either the stable or hydrate forms, is assessed without any information regarding the hydrate. DRIFTS was able to provide information on hydration without a standard sample and evaluate the physical stability focusing on the qualification of hydration in the early stages.

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