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Research Article

The Effect of Microcrystalline Cellulose Crystallinity on the Hydrophilic Property of Tablets and the Hydrolysis of Acetylsalicylic Acid as Active Pharmaceutical Ingredient Inside Tablets

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Abstract. The crystal structures of active pharmaceutical ingredients and excipients should be strictly controlled because they influence pharmaceutical properties of products which cause the change in the quality or the bioavailability of the products. In this study, we investigated the effects of microcrystalline cellulose (MCC) crystallinity on the hydrophilic properties of tablets and the hydrolysis of active pharmaceutical ingredient, acetylsalicylic acid (ASA), inside tablets by using tablets containing 20% MCC as an excipient. Different levels of grinding were applied to MCC prior to tablet formulation, to intentionally cause structural variation in the MCC. The water penetration and moisture absorbability of the tablets increased with decreasing the crystallinity of MCC through higher level of grinding. More importantly, the hydrolysis of ASA inside tablets was also accelerated. These results indicate that the crystallinity of MCC has crucial effects on the pharmaceutical properties of tablets even when the tablets contain a relatively small amount of MCC. Therefore, controlling the crystal structure of excipients is important for controlling product qualities.

KEYWORDS: crystallinity; hydrolysis; hydrophilic property; microcrystalline cellulose.

INTRODUCTION

It is well-known that the crystal structure of an active pharmaceutical ingredient affects its solubility, moisture absorbability, and stability, which in turn influence the bioavailability of pharmaceutical products (1–5). Therefore, the crystal structures of active pharmaceutical ingredients are, in general, strictly controlled in the manufacturing process of pharmaceutical products. However, the crystal structures of excipients also affect pharmaceutical properties of products. For example, Ando *et al.* revealed that the amorphous content of sucrose changed the hardness and water penetration of tablets stored for a certain period (6). Sebhatu *et al.* reported that tablets containing lactose had different hardness dependant on the amount of amorphous lactose (7). Consequently, it is most likely that the crystallinity of excipients can affect pharmaceutical properties of products, and so should be, in turn, strictly controlled in the manufacturing process.

In our previous study, we revealed that a decrease in the crystallinity of microcrystalline cellulose (MCC), a well-used pharmaceutical excipient, made it possible to pack the tablet tightly, which resulted in a delay in dissolution (8). We also reported that the development of the amorphous component increased the moisture absorbability of tablets (9). The variation of the crystalline structure of MCC also affects the hardness and dissolution profile of tablets (10). The disordered amorphous structure of MCC can bind the water molecule more strongly than the crystalline structure (11, 12). The variations in the affinity to water molecules related to crystallinity of MCC induce changes in pharmaceutical properties of products. In fact, it is known that the crystallinity of MCC can be decreased by some manufacturing processes (e.g., the grinding process and compression process) (13, 14). The tablets used in above-cited studies were constructed mainly from MCC, using 80–90% of MCC (8–10). While the analysis of such MCC tablets provides useful background information about variation in the supermolecular structure of MCC, the detailed mechanism, especially for actual pharmaceutical tablets, containing a much smaller amount of MCC, is not fully understood yet.

In this study, tablets containing 20% of MCC, 20% of an active pharmaceutical ingredient (either acetaminophen (AAP) or acetylsalicylic acid (ASA)), and 60% of other excipients were used to investigate the actual effects of MCC crystallinity on the pharmaceutical properties of tablets. MCC was ground before tablet formulation to intentionally cause a decrease in crystallinity. The water penetration into and moisture absorbability of tablets increased with grinding time, i.e., increased with the decrease in the crystallinity of MCC. ASA

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is a moisture-sensitive ingredient and hydrolyzed to salicylic acid in conditions of high humidity (15). The hydrolysis of ASA inside tablets was accelerated by the decrease in the crystallinity of MCC. That is to say, the decrease in the crystallinity of MCC decreased the stability of tablets which is one of the most important properties. These results indicate that the crystallinity of MCC has crucial effects on the pharmaceutical properties of products even if the tablets contain a relatively small amount of MCC.

MATERIALS AND METHODS

Materials

Acetaminophen (AAP), 4-acetamidophenol, was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 2-Acetylsalicylic acid (ASA) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). MCC, CEOLUS® (PH-101), was purchased from Asahi Kasei Chemicals Co. (Tokyo, Japan). Anhydrous dibasic calcium phosphate (DCPA), Fujicalin®, was purchased from Fuji Chemical Industry Co., Ltd. (Toyama, Japan). Their chemical structures are shown in Fig. 1. Magnesium stearate (Mg-St) was purchased from Taihei Chemical Industrial (Osaka, Japan). All reagents used as formulation components were used without further purification. All regents used for mobile phase of liquid chromatography were high-performance liquid chromatography grade. The other reagents were analytical grade.

Sample Preparation

AAP tablets containing AAP, MCC, DCPA, and Mg-St for the water penetration analysis and the moisture absorbability analysis were prepared as follows: AAP (20 wt%), unground MCC or ground MCC (20 wt%), DCPA (60 wt%), and Mg-St (0.2 wt%) were mixed. Ground MCC samples were prepared by grinding for 20, 40, and 60 min, respectively, with a vibration sample mill, TI-100 (Cosmic Mechanical Technology Co., Ltd., Fukushima, Japan), fitted with a porcelain rod. Subsample of the mixed powder (200 mg) was compressed to make a set of 8-mm diameter tablets with a manual tableting machine, HANDTAB-100 (Ichihashi-Seiki Co., Ltd., Kyoto, Japan), at a fixed pressure level, and several tablets were made per grinding condition. The 10 kN of pressure was gradually applied to the upper punch to circumvent the generation of unwanted frictional heat. Pressure was released immediately after reaching 10 kN. The samples were handled at approximately 25°C and 75% relative humidity.

ASA tablets containing ASA, MCC, DCPA, and Mg-St, for the stability studies, were prepared as follows: ASA (20 wt%), unground MCC or ground MCC (20 wt%), DCPA (60 wt%), and Mg-St (0.2 wt%) were mixed. Ground MCC samples were prepared by grinding for 90 min with a vibration sample mill fitted with a porcelain rod. A set of tablets was prepared by the same process described above. The samples were handled at approximately 25°C and 40% relative humidity.

X-ray Powder Diffraction Analysis

X-ray powder diffraction (XRD) profiles of the ground MCC were recorded with an X-ray diffractometer, RINT-ULTIMA III (Rigaku Co., Tokyo, Japan). The diffracted intensity under Cu Ka radiation (40 kV and 50 mA) was measured with a scan range of 5–40° and scan step of 0.02° at scan rate of 2°/min.

Water Penetration Analysis

Tablets were placed on a 55-mm diameter filter paper soaked with 500 μL of water, and then, the amount of time for water to penetrate the entire tablet was measured (N=3). The end point of penetration was determined by visual observation. Penetration time per unit volume was defined as follows: Penetration time per unit volume (s/mg/mm^3)=[the time for water to penetrate entire tablet (s)]/[the density of the tablet (mg/mm^3)]. The densities of the tablets used in water penetration analysis were calculated in advance using their weights, diameters, and thicknesses. The control tests were also performed using chloroform instead of water (N=3).

Moisture Absorption Analysis

Tablets were dried with a vacuum drying oven, DP23 (Yamato Scientific Co., Ltd., Tokyo, Japan), for 48 h at 25°C. Then, each tablet underwent moisture absorption analysis by a moisture sorption analyzer, IGAsorp (Hiden Isochema Ltd., Warrington, UK) (N=1). The increase in weight caused by the transient moisture absorption was monitored at a fixed temperature 25°C and 95% relative humidity.

X-ray Computed Tomography

X-ray computed tomography (CT) images of the tablets were scanned with a SKY SCAN 1172 (Bruker micro-CT, Kontich, Belgium) equipped with a Cu tube, with a spatial resolution of 5.5 μm and a rotation step of 0.40° (N=1). The X-ray source was operated using a tube voltage of 60 kV and current of 100 μA.

Stability Study

ASA tablets were stored in an airtight container kept at a relative humidity of 75% using a saturated solution of sodium chloride. The container was stored in an incubator kept at 40°C for 4 weeks. Tablets were sampled every 7 days and assayed for amounts of the hydrolysate product of ASA, i.e., salicylic acid, using liquid chromatography (N=3).

The assay of salicylic acid by liquid chromatography was performed as follows: Each tablet was suspended in a mixture of water and methanol (1:3), and the concentration of the ASA was about 1 mg/mL. The suspension was filtered and then assayed by liquid chromatography. An octadecylsilyl column, XBridge C18 (2.5 μm in particle size, 4.6 mm in diameter × 5 cm in length, Waters) was used. The mobile phase was a mixture of 0.1% phosphoric acid aqueous solution and acetonitrile (1:4). The detector was an ultraviolet absorption photometer (wavelength 254 nm). An amount of salicylic acid was calculated using the following formula: Amount (%) of salicylic acid=[Peak area of salicylic acid]/([Peak area of ASA]+[Peak area of salicylic acid])×100.

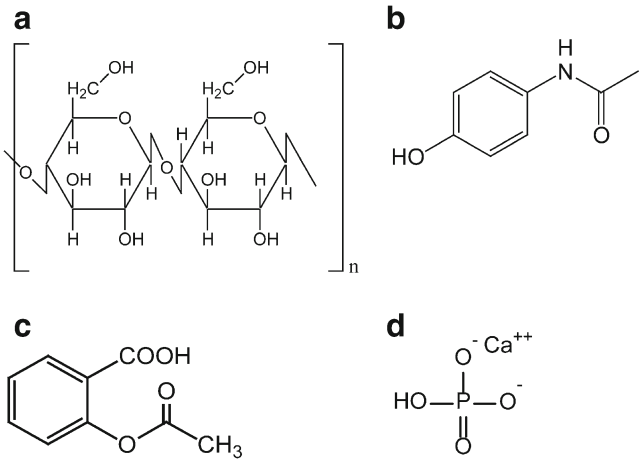

Fig. 1. Chemical structures of **a** cellulose, **b** acetaminophen (AAP), **c** acetylsalicylic acid (ASA), and **d** anhydrous dibasic calcium phosphate (DCPA)

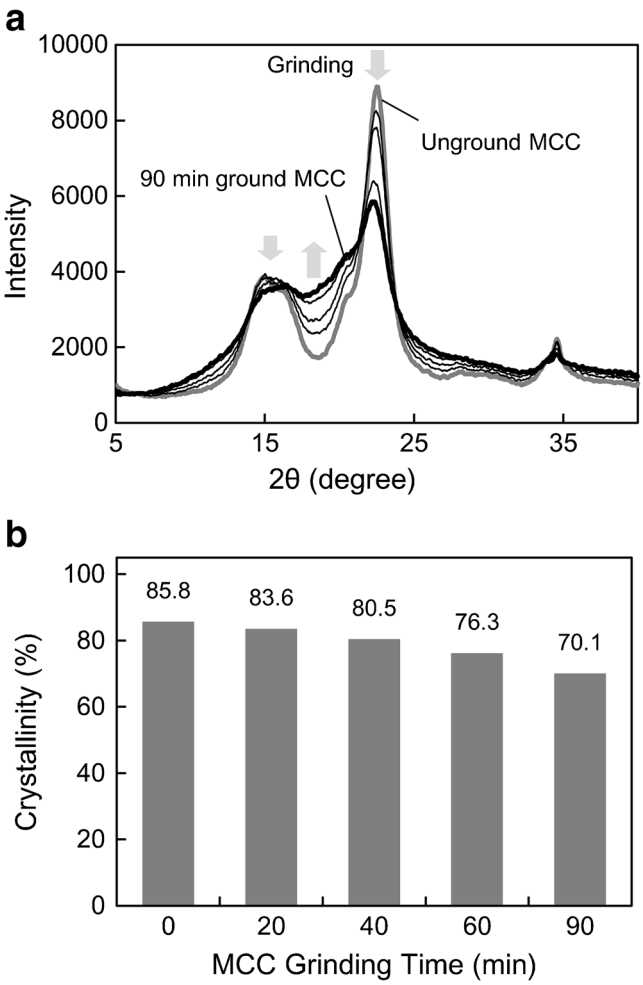

Fig. 2. **a** XRD patterns of MCC powder prepared under varying grinding time and **b** crystallinity of MCC powder calculated from the XRD patterns

RESULTS

Crystallinity of Ground Microcrystalline Cellulose

The change in MCC crystallinity induced by grinding was investigated by XRD. The XRD patterns of the MCC powder samples are shown in Fig. 2a. The crystalline structure of cellulose provides distinct peaks at 15°, 16.4°, and 22.5°. While it is not clearly observed in the XRD pattern, it is known that the amorphous component also generates a halo peak around 21° (16). In our results, the peaks at 15°, 16.4°, and 22.5°gradually decreased and broadened but, in contrast, the halo peak increased with grinding time. The crystallinities of MCC powder calculated from the XRD patterns are shown in Fig. 2b. Note that the crystallinity was calculated as the ratio of the crystalline and amorphous components using a curve-fitting method (16). It can be seen from Fig. 2b that the crystallinity of MCC gradually decreased with grinding time.

Water Penetration into Tablets

Water penetration into tablets was investigated using water penetration analysis. The water and chloroform penetration times into AAP tablets containing unground MCC or 20-, 40-, or 60-min ground MCC are shown in Fig. 3a. The densities of tablets used for the water penetration analysis are shown in Fig. 3b for reference. It should be noted that the water penetration time gradually decreased with the increase in grinding time despite no significant variation in tablet density. On the other hand, in the control tests using chloroform which has little interaction with MCC, the chloroform penetration times were nearly equal among samples. These results indicate that the grinding of MCC provided a substantial level of variation in the water penetration property of the tablet.

Moisture Absorbability of Tablets

Figure 4 represents the variations in tablet weight during the moisture absorption analysis. When exposed to a controlled atmosphere, each tablet started to absorb water molecules to increase its relative weight. Additionally, the relative weight obviously increased more rapidly with grinding time, suggesting that the grinding of MCC led to an increase in moisture absorbability of the tablet. It is well known that the water absorption by a tablet is often affected by its physical features, for example, tablet porosity (8). Therefore, we investigated the tablet porosity by X-ray CT. Figure 5a shows top view images of the inside of tablets by X-ray CT. X-ray CT generates a three-dimensional image of the inside of an object from a large series of two-dimensional X-ray images. The gray level of each pixel in a CT image corresponds to the X-ray attenuation, which is caused by the density and composition of the material (17). Thus, the X-ray CT image reflects any differences in tablet density. The X-ray CT images in Fig. 5a show no significant differences in patterns. Figure 5b shows the porosities estimated by binarization of three-dimensional images. Note that the porosity, defined as the ratio of pixels having binary value 0 or 1, was calculated after the binarization with a threshold set to be one sixth of the maximum intensity of the image. Our results show no significant differences in porosities. This lack of difference in CT patterns and porosities can be interpreted to mean that the increase in the

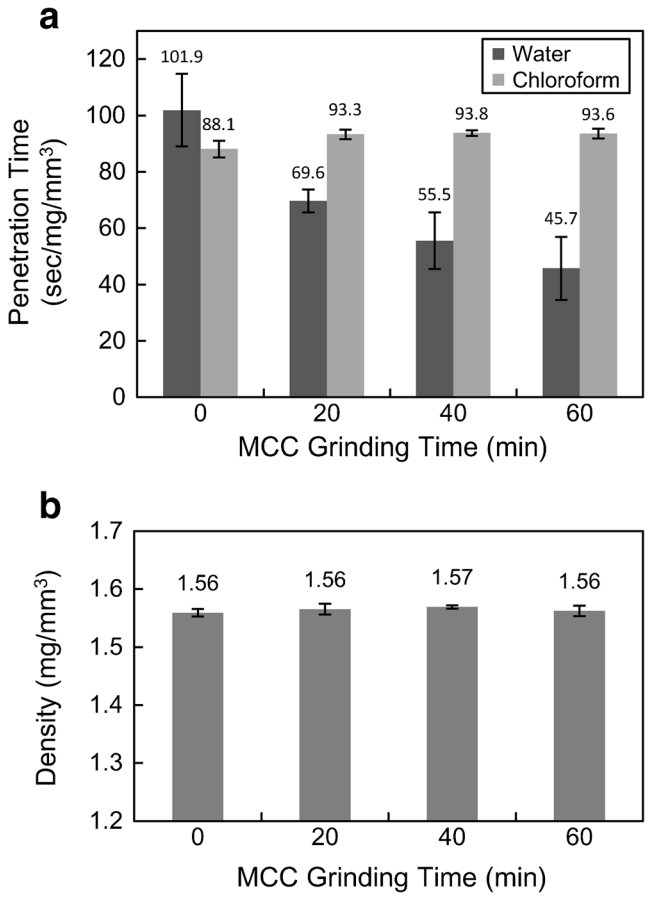

Fig. 3. **a** Penetration time of water (*dark gray*) and chloroform (*light gray*) into the AAP tablets containing AAP, unground or ground MCC, DCPA, and Mg-St and **b** densities of these tablets

grinding time did not provide any distinguishable difference in tablet porosity. In fact, the corresponding weights and volumes of the tablets also provided less significant differences (data not shown). These results indicate that the variation of the water absorption behaviors shown in Fig. 4 was induced by the grinding of MCC and not by the porosity of tablets.

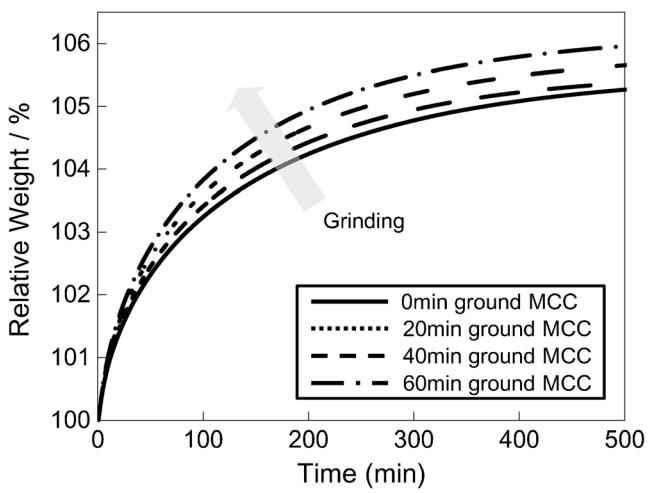

Fig. 4. Change in the relative weight of the AAP tablets containing MCC ground for 0 (*continuous line*), 20 (*dotted line*), 40 (*dashed line*), and 60 min (*dashed dotted line*) under 25°C and 95% relative humidity

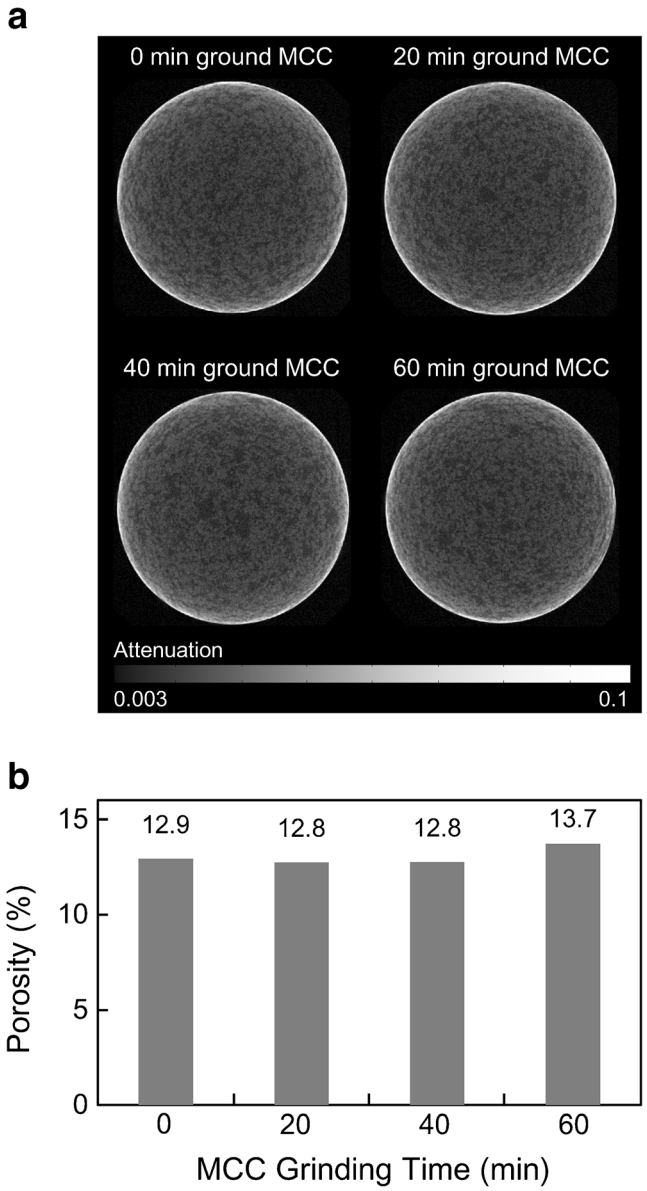

Fig. 5. **a** X-ray CT images of the AAP tablets containing MCC prepared under varying grinding time and **b** the porosities of tablets estimated by three-dimensional image of X-ray CT

Stability of Tablets

Figure 6 shows the amount of salicylic acid in the tablets plotted against storage time. When ASA tablets prepared using unground MCC or 90-min ground MCC were stored at 40°C and 75% relative humidity, all tablets showed an obvious increase in the salicylic acid content. Of note is that the development of salicylic acid was especially pronounced for the tablets containing 90-min ground MCC. The result in Fig. 6 shows that the hydrolysis of ASA inside tablets under high temperature and humidity conditions was accelerated with the grinding time of MCC.

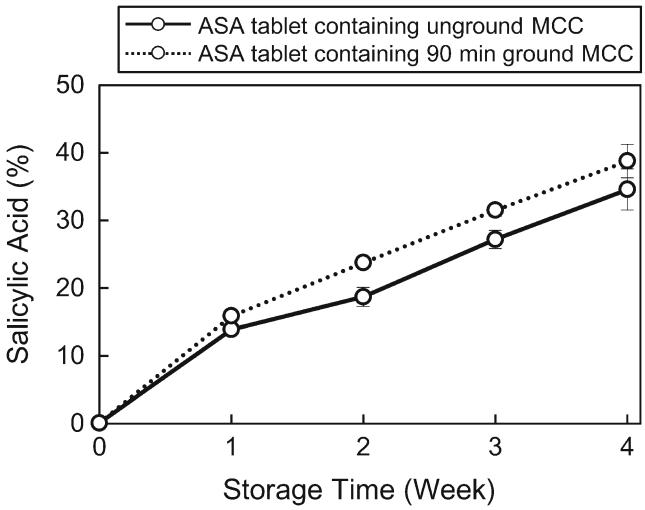

Fig. 6. Amounts of salicylic acid in the ASA tablets containing unground MCC (*continuous line*) and 90-min ground MCC (*dotted line*) during 4-week storage at 40°C and 75% relative humidity

DISCUSSION

Effect of Microcrystalline Cellulose Crystallinity on the Hydrophilic Property

In this study, we applied different levels of grinding to MCC prior to tablet formulation, to intentionally cause structural variation in the MCC (18). As shown in Fig. 2, the XRD patterns of the MCC powder samples and their crystallinities show that the grinding induced the disintegration of the crystalline structure and subsequent development of amorphous component.

In the first part of the study, we investigated the effect of crystallinity of MCC on the hydrophilic properties of the tablets. Water penetration time into tablets shown in Fig. 3a gradually decreased with the increase in grinding time of MCC. The formulation components other than MCC did not undergo any processes before tablet formulation. Therefore, we interpreted this to mean that the variation of the crystalline structure of MCC predominantly influences the water penetration behavior of the tablets. The grinding substantially induces variation in the polymer structure of MCC, which eventually decreases the crystallinity of MCC and then generates an amorphous structure. We believe that the disordered amorphous structure tends to bind water via the development of hydrogen bonds between the MCC and water molecules, which, in turn, accelerates the penetration of water into the tablets. This hypothesis is supported by the smaller variation in chloroform penetration time. Chloroform has less polarity and hence less molecular interaction with MCC. Consequently, it is likely that grinding induces the disintegration of the MCC crystalline structure and ultimately provides an increase in water penetration of the tablets.

The variation of the crystalline structure of MCC also influences the moisture absorbability of the tablets. In Fig. 4, one can find that the relative weight obviously increased more rapidly with grinding time, i.e., with the decrease in crystallinity of MCC. As shown in Fig. 5, the grinding did not provide any distinguishable difference in tablet porosity. These results indicate that the increase in moisture absorbability was induced by the crystallinity of MCC and not by physical features of tablets. Accordingly, it is likely that the acceleration of moisture absorption occurs by the following mechanism: The grinding process induces a decrease in crystallinity of MCC and generates an amorphous component. As discussed above, the disordered amorphous component tends to bind the water molecule more strongly via the development of hydrogen bonds between the MCC and water molecules, which accelerates the moisture absorption of tablets.

Effect of Microcrystalline Cellulose Crystallinity on the Stability of Tablets

So far, we have revealed that the molecular level variation of MCC affected on the hydrophilic properties on tablets containing only 20% MCC. It is also useful to know how such a variations in the excipient affects the stability of an active pharmaceutical ingredient inside the tablets. In this study, we investigated the hydrolysis of ASA, moisture-sensitive ingredient, as a kind of quantitative index of stability. The result in Fig. 6 shows that ASA was hydrolyzed into salicylic acid under high temperature and humidity condition. More importantly, development of salicylic acid was especially pronounced for the tablets containing ground MCC which has low crystallinity.

It is likely that the decrease in stability occurred due to the following mechanism: The water penetration and moisture absorbability of tablets containing MCC are affected by the crystallinity of MCC. During storage under high humidity conditions, the tablets were exposed to atmospheric moisture. The moisture absorption by tablets was accelerated by the decrease in MCC crystallinity, which led to the acceleration of water penetration into tablets. ASA inside the tablets containing more amorphous MCC made contact with water molecule more often and eventually the hydrolysis of ASA was accelerated.

Consequently, these results reveal that molecular level alternation of MCC can provide possible variations in the pharmaceutical properties of the tablets even if the tablets contain only 20% MCC. Though crystal structures of active pharmaceutical ingredients are strictly controlled during the manufacturing processes of pharmaceutical products, those of excipients are often overlooked. However, we have shown that the variation of crystalline structure of MCC has crucial effects on not only the water penetration and moisture absorbability but also stability which is one of the most important properties of pharmaceutical products, when the tablets contain a relatively small amount of MCC. Therefore, the control of crystal structure of excipients during the manufacturing process is also important for the control of product quality.

CONCLUSION

In this study, we investigated the effect of crystallinity of MCC on the hydrophilic properties of tablets and the hydrolysis of active pharmaceutical ingredient inside tablets by using tablets containing 20% MCC. The stronger interaction between MCC and water molecules induced by the decrease in the crystallinity of MCC increased the water penetration

and moisture absorbability of tablets. In addition, the increases in the water penetration and moisture absorbability eventually accelerated the hydrolysis of moisture-sensitive ingredient inside tablets during storage. These results in this study indicate that the crystallinity of MCC has crucial effects on the pharmaceutical properties of products even when the tablets contain only 20% MCC.

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