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The Effect of Mechanical Grinding on the Formation and Crystallinity Changes of the Inclusion Complex of Acetaminophen and β-Cyclodextrin*

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Abstract. The effect of mechanical grinding on the physicochemical properties of acetaminophen in the presence of three additives, α- or β-cyclodextrin and microcrystalline cellulose, was studied by using TLC, powder X-ray diffraction analysis, infrared spectroscopy and differential scanning calorimetry. The results indicate that the crystallinity of physical mixtures of acetaminophen and the described additives decreased with increased grinding time and formed an amorphous state when mixtures containing α- or β-cyclodextrin were ground with acetaminophen. We also found that the acetaminophen molecules could be included step-by-step into the cavity of β-cyclodextrin molecules and formed an amorphous inclusion complex. α-Cyclodextrin and microcrystalline cellulose did not form an inclusion complex with acetaminophen, but acted only to decrease the crystallinity of the ground mixtures. The mechanical grinding efficiency for acetaminophen was improved in the order of β-cyclodextrin ≫ α-cyclodextrin > microcrystalline cellulose.

Key words. Mechanical grinding, ball mill, α- or β-cyclodextrin, microcrystalline cellulose, crystallinity, amorphous, inclusion complex.

1. Introduction

Recently, it has been found that mechanical grinding can be employed as a means of reducing the particle size of solid drugs to improve the dissolution rate and to change molecular properties of medicinals such as crystallinity, the phase transition of polymorphs, and molecular interaction in the solid state [1–4]. Grinding efficiency is not only strengthened by the mechanical stress but also improved by the addition of additives [5, 6].

Cyclodextrins have raised considerable interest and attention, due to their specific chemical modification and ability to form an inclusion complex thus improving the solubility, stability, and bioavailability of many drugs [7, 8]. In our laboratory, we have reported that cyclodextrins can not only improve the dissolution rate of warfarin (>1000-fold) but can also control the release rate of warfarin from an inclusion complex resulting in the prolongation of the prothrombin time [9, 10]. We also found that water-insoluble drugs can be dissolved without recrystallization from an amorphous state in the ground mixture by co-grinding with β-cyclodextrin

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[11–13]. A few studies have been published about the formation of cyclodextrin inclusion complexes obtained by grinding [14–16].

Acetaminophen is a widely used non-salicylate analgesic and antipyretic drug. The solubility and stability of acetaminophen have been improved by the addition of cyclodextrins, due to the formation of an inclusion complex in aqueous solution [17–19]. In the present study, we used a mechanical grinding method to investigate whether or not the inclusion complexes of acetaminophen and cyclodextrins were formed when both materials were milled together in the solid state. The effect of grinding time on the physicochemical properties of acetaminophen in the ground mixtures was studied. Microcrystalline cellulose was also used as a comparison compound.

2. Experimental

2.1. MATERIALS

Acetaminophen (pharmaceutical grade) was purchased from Seven Star Pharm. Co. Ltd., Taiwan, R.O.C. α- and β-Cyclodextrins were obtained from Nihon Shokuhin Kako Co. Ltd., Tokyo, Japan. Microcrystalline cellulose (Avicel 101) was purchased from Asahi Kasei KoGyo Co., Tokyo, Japan. All other materials and reagents were of analytical reagent grade.

2.2. PREPARATION OF GROUND MIXTURES

The ground mixtures of acetaminophen and α- or β-cyclodextrin in a 1 : 1 molar ratio were respectively prepared by grinding the materials in a ceramic ball mill for 24 hours. During the grinding process, samples were withdrawn at prescribed intervals (0, 0.5, 1.0, 1.5, 3.0, 5.0, 7.0, and 24 hours) for further examination. Ground mixtures of acetaminophen with Avicel (weight ratio = 1 : 1) were also prepared.

2.3. PREPARATION OF FREEZE-DRIED SAMPLES

Freeze-dried samples were prepared by dissolving α- or β-cyclodextrin and acetaminophen (molar ratio = 1 : 1) in water, afterwards these dissolved samples were freeze-dried.

2.4. IDENTIFICATION OF PHYSICAL MIXTURES, FREEZE-DRIED SAMPLES AND GROUND MIXTURES

The physical mixtures, freeze-dried samples and ground mixtures were examined using X-ray powder diffractometry (Geigerflex D/Max-IIIA, Rigaku Denki, Japan; Ni filter, Cu K_α radiation, 25 kV, 15 mA, scanning speed, 2θ/min), differential scanning calorimetry (DSC-1090, Dupont, USA; open pan system in N_2 gas flow, heating rate: 10°C/min) and infrared spectrophotometry by the KBr disc method (IR-580, Perkin-Elmer, USA). TLC was performed on glass plates coated with silica 60 (F_{254} (Merck)) to examine the stability of acetaminophen in the mixtures. The plates were developed in a mobile phase of chloroform : ethanol : acetic acid (44 : 5 : 1) and the spots were detected by exposure to UV light and iodine vapor.

2.5. CRYSTALLINE TRANSFORMATION KINETIC STUDIES

The transformation kinetics of solid crystalline acetaminophen were studied after crystalline acetaminophen was ground with α- or β-cyclodextrin (molar ratio = 1 : 1) or Avicel (weight ratio = 1 : 1) by the DSC method. Physical mixtures of acetaminophen and additives were previously prepared by a light trituration in a glass mortar followed by placement of the mixture in a ball mill for grinding. At prescribed time intervals, transition energies of the ground samples were determined at the 168°C endothermic peak by DSC thermal analysis. The crystallinity changes of acetaminophen in the ground mixtures were directly calculated as follows [20]:

$$\text{Crystallinity (\%)} = \frac{\Delta H_t}{\Delta H_i} \times 100 \tag{1}$$

where ΔH_t is the heat of fusion determined at the prescribed grinding time and ΔH_i is the heat of fusion of the original crystalline acetaminophen. The measurements were taken five times for each sample and an average was obtained.

3. Results and Discussion

A number of studies have focused on the stability of drugs in the solid state due to mechanical activation [21–23], because the mechanical grinding force creates many new surface areas and transfers the energy to these created surface areas, leading to a change in crystallinity, polymorph, solubility and stability of the solid drug. In this study, however, no other spot except that corresponding to acetaminophen ($R_f = 0.68$) and cyclodextrin was found in the thin-layer chromatograph plates, indicating that no decomposed compound was produced during grinding. Moreover, the freeze-dried sample containing β-cyclodextrin was shown to be an inclusion complex, as described in our previous study [13], but the freeze-dried sample containing α-cyclodextrin was found to be a physical mixture of acetaminophen and α-cyclodextrin.

Figure 1 shows the powder X-ray diffraction patterns of crystalline acetaminophen, the three additives, and ground mixtures obtained at various times. It is evident that the peak intensity for all the ground mixtures containing α- or β-cyclodextrin considerably decreased with an increase in the grinding time. There was no reflection peak on the diffractogram after 24 hours of grinding, suggesting that both the crystalline form of acetaminophen and the cyclodextrins were transformed into an amorphous state by grinding. But stronger intensity peaks were still found in freeze-dried samples containing β-cyclodextrin (Figure 1-A) and in acetaminophen alone [13]. Moreover, a decreased intensity of the acetaminophen peaks was also found in the ground mixtures containing Avicel, but it did not become amorphous even after 24 hours of grinding. No changes in the peak positions from the X-ray diffractogram were observed. This suggests that the grinding efficiency was improved by the addition of α- or β-cyclodextrin.

DSC thermograms of acetaminophen, α-, β-cyclodextrin, physical mixtures, and ground mixtures are shown in Figures 2 and 3. A sharp endothermic peak at 168°C was observed for both the non-ground acetaminophen crystal and the ground

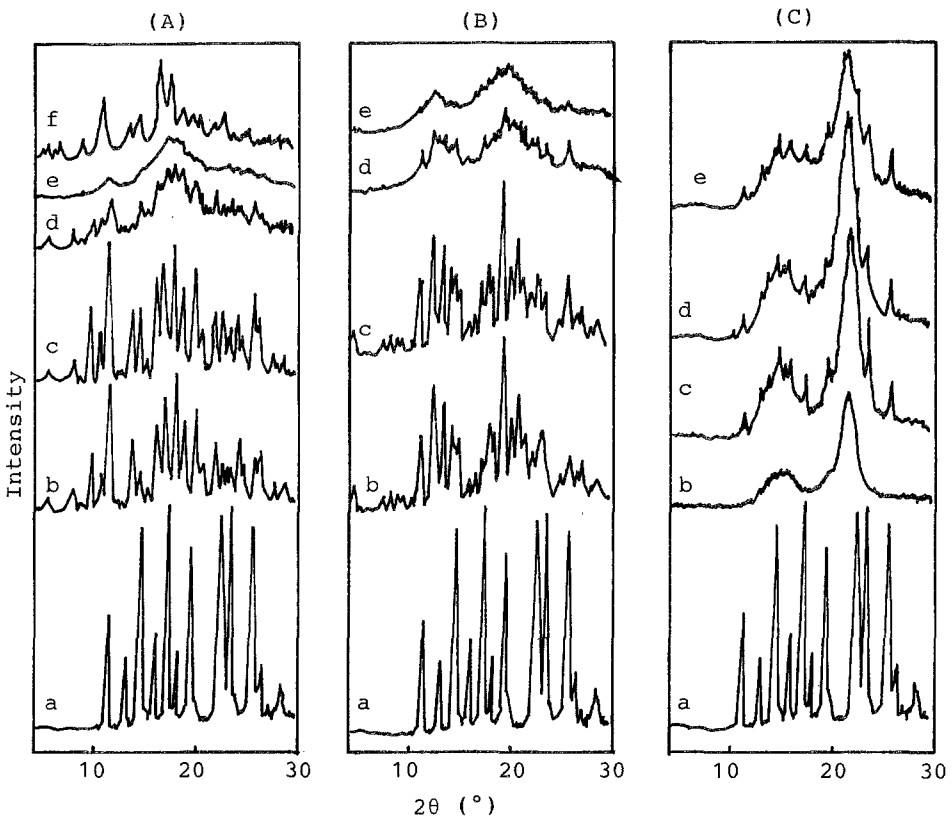

Fig. 1. The powder X-ray diffractograms of acetaminophen, additives and their ground mixtures. Key: (A) β-cyclodextrin system; (B) α-cyclodextrin system; (C) microcrystalline cellulose system – (a) pure acetaminophen; (b) additive; (c) physical mixture; (d) ground mixture (grinding for 3 hr); (e) ground mixture (grinding for 24 hr); (f) freeze-dried sample.

acetaminophen, due to fusion of the acetaminophen (Figure 2). This indicates that crystalline acetaminophen in and of itself was not affected by grinding. This result agrees with the data of the X-ray diffractogram. Figure 3 shows the effect of grinding on the DSC thermograms of acetaminophen-additive mixtures. It is obvious that the endothermic peak for acetaminophen at 168°C decreased with decreasing grinding time. After 24 hours of grinding, this endothermic peak around 168°C disappeared for both the α- and β-cyclodextrin mixtures, whereas the ground mixtures containing Avicel displayed a slightly decreased endothermic peak. Two possibilities may explain this disappearance phenomenon at 168°C in the DSC thermograms. One is that the inclusion complex was formed as it was in the freeze-dried sample without any endothermic peak at 168°C. Another possibility is that the ground mixture became amorphous.

Figure 3 also indicates that acetaminophen melted at 168°C and decomposed initially in the same range. However, the DSC curve of the physical mixture before grinding exhibited a small exothermic peak after melting (Figures 3-A and B). This

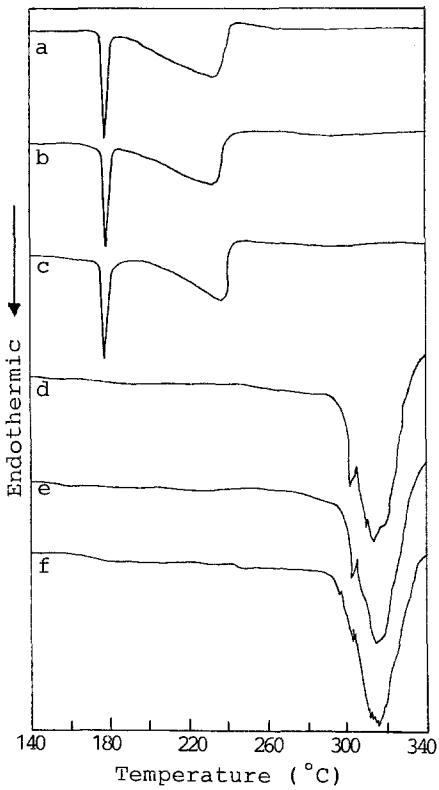

Fig. 2. DSC thermograms of acetaminophen and β-cyclodextrin. Key: (a–c): acetaminophen; (d–f): β-cyclodextrin. (a, d), pure material; (b, e), grinding for 3 hr; (c–f), grinding for 24 hr.

exothermic peak might be attributed to complex formation due to heating [24]. Another new exothermic peak which appeared before the melting peak was also observed in the DSC thermograms of acetaminophen-β-cyclodextrin ground mixtures, but it did not appear in the DSC curve of acetaminophen-α-cyclodextrin ground mixtures. This new exothermic peak before the melting point was due to the molecular interaction between the free acetaminophen molecule and the inclusion complex [25].

Figure 4 shows the IR spectra of crystalline acetaminophen, the additives, ground mixtures, and freeze-dried samples. In the case of a freeze-dried sample containing β-cyclodextrin, the C—O stretch of the phenolic group of acetaminophen was shifted from 1226 cm^{-1} to 1245 cm^{-1}, suggesting the dissociation of the intermolecular hydrogen bonds of acetaminophen through inclusion complexation. The C=C stretch of the benzene ring of acetaminophen at 1612 cm^{-1} disappeared when the acetaminophen-β-cyclodextrin inclusion complex formed. This clearly demonstrates that the phenolic group was first included into the cavity of β-cyclodextrin, resulting in a disappearance of the C=C band of the benzene ring of acetaminophen. The amido group frequency of acetaminophen (1568 cm^{-1}) was also shifted to a lower frequency (1555 cm^{-1}). This might be attributed to intermolecular hydrogen

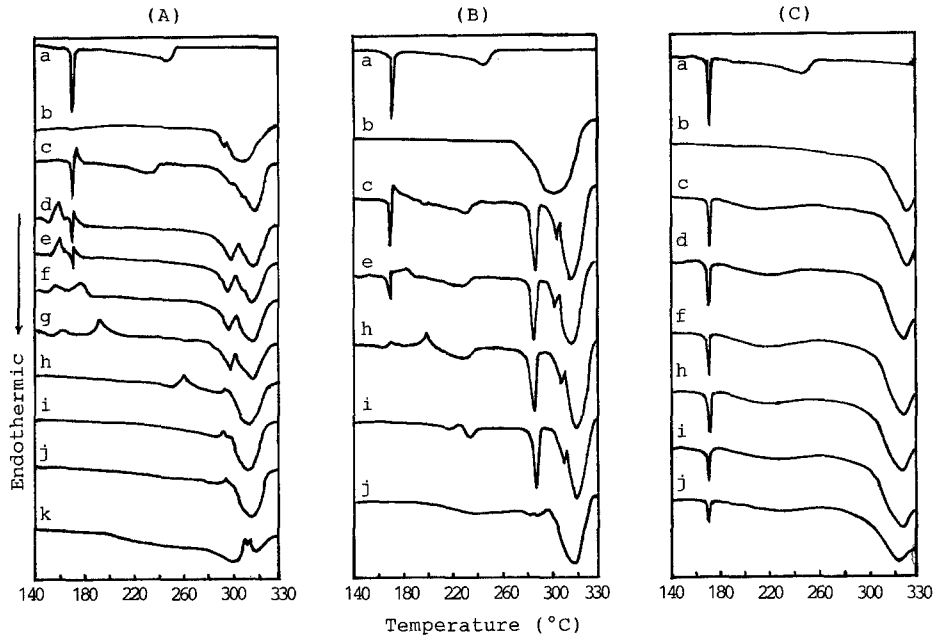

Fig. 3. DSC thermograms of acetaminophen, additives and their ground mixtures. Key: (A) β-cyclodextrin system; (B) α-cyclodextrin system; (C) microcrystalline cellulose system – (a) pure acetaminophen; (b) additive; (c) physical mixture; (d) ground mixture (grinding for 0.5 hr); (e) ground mixture (grinding for 1.0 hr); (f) ground mixture (grinding for 1.5 hr); (g) ground mixture (grinding for 2.0 hr); (h) ground mixture (grinding for 3.0 hr); (i) ground mixture (grinding for 7.0 hr); (j) ground mixture (grinding for 24 hr); (k) freeze-dried sample.

bonding between the amido group of acetaminophen and a hydroxyl group of cyclodextrin. Similar changes in IR spectra of inclusion complex have also been observed in other studies [14, 15, 26].

Thus, the postulated structure of the acetaminophen-β-cyclodextrin inclusion complex may be as shown in Figure 5. The freeze-dried sample containing α-cyclodextrin did not form an inclusion complex, presumably because of the small cavity size of α-cyclodextrin. Figure 4 also shows that with increasing grinding time, some spectra of acetaminophen in the ground mixtures with β-cyclodextrin, as described above, slowly shifted to the spectral positions of the inclusion complex. After 24 hours of grinding, the IR spectra of the mixtures containing β-cyclodextrin was the same as the IR spectra of the freeze-dried inclusion complex. This result clearly implies a possibility of inclusion complex formation by mechanical grinding of the physical mixture. However, in the acetaminophen-α-cyclodextrin system or the acetaminophen-Avicel system the IR spectra of all the ground mixtures were similar to the IR spectra of acetaminophen. This indicates that α-cyclodextrin and Avicel do not form inclusion complexes by grinding or freeze drying although the thermal behavior of the ground mixtures containing α-cyclodextrin was similar to that of the ground mixtures containing β-cyclodextrin. The results are considered to be due to the small cavity size which cannot include acetaminophen molecules.

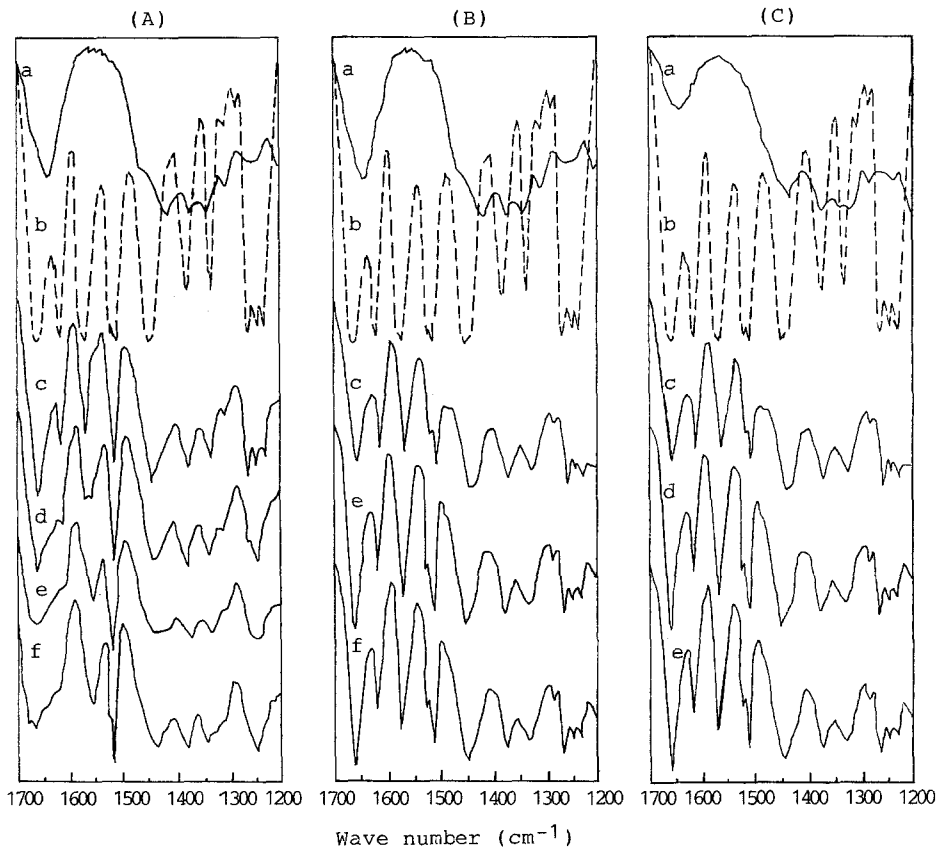

Fig. 4. IR spectra of acetaminophen, the additives and their ground mixtures. Key: (A) β-cyclodextrin system; (B) α-cyclodextrin system; (C) microcrystalline cellulose system – (a) additive; (b) pure acetaminophen; (c) physical mixture; (d) ground mixture (grinding for 3 hr); (e) ground mixture (grinding for 24 hr); (f) freeze-dried sample.

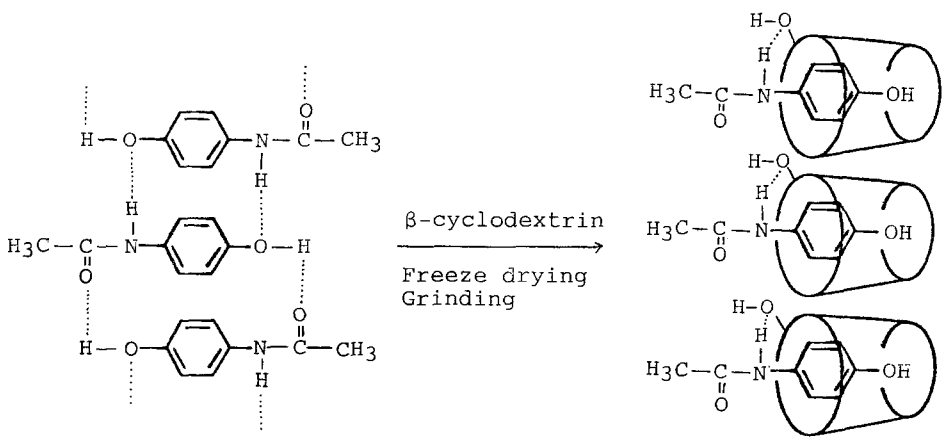

Fig. 5. Scheme of the postulated structure for the formation of the acetaminophen–β-cyclodextrin inclusion complex.

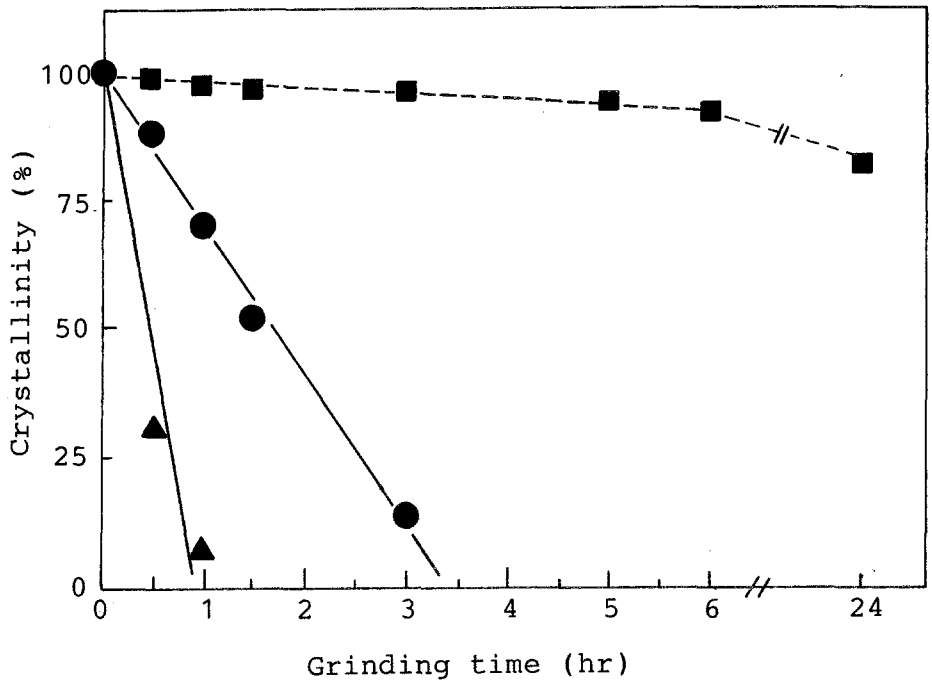

Fig. 6. Effect of grinding time on the crystallinity changes of acetaminophen in the presence of the different additives. Key: ●, α-cyclodextrin; ▲, β-cyclodextrin; ■, microcrystalline cellulose.

Since acetaminophen was converted to the amorphous state upon grinding with α- or β-cyclodextrin, as the above results show, in order to examine the solid crystal state transformation process, the transformation kinetics of acetaminophen when co-grinding with α- or β-cyclodextrin or Avicel was studied by using the DSC method. The fraction transformed from the crystalline state to the amorphous state at a prescribed grinding time was estimated by Equation (1), as the relative enthalpy change is considered to be a measure of the apparent degree of crystallinity. The effect of grinding time on the crystallinity changes of acetaminophen with different additives is shown in Figure 6. It is obvious that the degree of crystallinity of the ground mixture decreased with increasing grinding time and that acetaminophen became amorphous after a short grinding time with α- or β-cyclodextrin. The ground mixtures containing β-cyclodextrin need 1 hr of grinding time to transform the crystalline physical mixtures into an amorphous state, but it needs 3.5 hr of grinding time for the physical mixtures containing α-cyclodextrin to form an amorphous state. On the other hand, the degree of crystallinity decreased with the increase of grinding time to 80% only for the 24 hr ground mixtures containing Avicel. The results indicate that the ground mixtures containing α- or β-cyclodextrin more easily became amorphous than that of ground mixtures containing Avicel [27]. This might be because cyclodextrin crystals were pulverized more easily than Avicel in a short grinding time, resulting in the acetaminophen crystals being transformed more easily into the amorphous state by mechanical grinding. Moreover, we

also deduce that the ground acetaminophen in an amorphous state could be stabilized when included into the cavity of β-cyclodextrin by forming an inclusion complex with a lower chemical potential.

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