

Original Article

## Co-grinding with Cyclodextrin as a Nanoparticle Preparation Method of a Poorly Water Soluble Drug

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### Abstract

The method of co-grinding with cyclodextrins (CDs) was applied to a poorly water soluble drug, ONO-8713 (solubility; 0.92  $\mu\text{g}/\text{ml}$  in  $\text{H}_2\text{O}$  at 25 °C) as a method to prepare nanoparticles. ONO-8713 was co-ground with various CDs in a vibration mill.  $\alpha$ -Cyclodextrin,  $\beta$ -CD,  $\gamma$ -CD, CD derivatives and some sugars were used as co-grinding additives. Suitable moisture content in the co-grinding system was required to achieve maximum nanoparticle yield. When ONO-8713 was co-ground with  $\beta$ -CD (molar ratio of  $\beta$ -CD:drug = 5:1) at 12% moisture, 85% of drug recovered as nanoparticles with a mean particle size of 120 nm. Nanoparticle yield achieved 90% when hydroxypropyl- $\beta$ -CD was used as a co-grinding additive. It was found that the amount of drug nanoparticles depended on the characteristics of CDs. This phenomenon was probably due to the difference in the cavity size of CDs along with the variation of substitution groups that affected the interaction between CDs and drug, and the affinity between CD and drug molecules. Zeta potential analysis suggested that CD would form a layer covering on the particle surface and alter the charge of the particles, improving the stability and total yield of the nanoparticle.

### Introduction

Particle engineering technology has drawn much attention and interest as the method for enhancing solubility and bioavailability of poorly water soluble drugs [1, 2]. Drug fine particles typically can be prepared by spray drying [2], rapid expansion of the supercritical solutions (RESS) method [3, 4] and grinding [5]. The grinding method shows several advantages because it can be performed easily and environmental friendly because it requires no organic solvents. However, grinding hydrophobic drugs usually causes an aggregation of drug particles and consequently results in the limitation of size reduction around 3  $\mu\text{m}$ . Size reduction to nanometer range should be carried out by other techniques.

The co-grinding technique has been employed for amorphization of drugs [6] and complex formation between drugs and host molecules (cyclodextrins [7], cholic acid [8], and deoxycholic acid [9]). Recently, co-

grinding with some additives was found to be a simple and effective method to prepare drug nanoparticles [10–14]. Cyclodextrins (CDs), mannitol or a combination of polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate [15] are examples of co-grinding additives. In previous papers [13, 14], we reported a formation of crystalline nanoparticles of pranlukast hemihydrate (mean particle size 192 nm) by co-grinding with  $\beta$ -CD. We also found that the moisture content in the co-grinding process significantly affected nanoparticle formation and there was an optimum range of moisture for nanoparticle formation. We proposed that the nanoparticle was formed due to the surface interaction between CD and drug, along with the CD network covering the particle surface.

In this report, the application of this method to another poorly water soluble drug was studied using ONO-8713 as a model drug. ONO-8713, a prostaglandin E2 antagonist, has a very low aqueous solubility (0.92  $\mu\text{g}/\text{ml}$  in  $\text{H}_2\text{O}$  at 25 °C) and poor bioavailability. With the intention of improving the aqueous solubility and bioavailability, we have applied the method of co-grinding

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using CDs and CD derivatives in an attempt to prepare crystalline nanoparticles of ONO-8713. Various types of CDs as well as non-CD additives were investigated.

## Experimental

### Materials

ONO-8713, (*E*)-3-[4-[[2-*N*-(furan-2-sulfonyl)-*N*-isobutylamino]-5-(trifluoromethyl)phenoxy] methyl]phenyl]propenoic acid (MW 523.53) was received as a gift from Ono Pharmaceutical Co. Ltd., Japan. The additives were obtained from sources as follows:  $\beta$ -CD and hydroxypropyl- $\beta$ -CD (HP- $\beta$ -CD) (degree of substitution: 4.6) from Nihon Shokuhin Kako Co. Ltd., Japan.  $\alpha$ -CD and  $\gamma$ -CD from Mercian Corporation, Japan. Heptakis(2,6-di-*O*-methyl)- $\beta$ -CD (DM- $\beta$ -CD) and heptakis(2,3,6-tri-*O*-methyl)- $\beta$ -CD (TM- $\beta$ -CD) from Tohshin Chemical Co. Ltd., Japan. Mannitol, glucose and sucrose from Nacalai Tesque, Inc., Japan. Microcrystalline cellulose (MCC) from Asahi Kasei Co., Japan. The water contents of intact  $\alpha$ -CD,  $\beta$ -CD,  $\gamma$ -CD, HP- $\beta$ -CD, DM- $\beta$ -CD, TM- $\beta$ -CD, mannitol, glucose, sucrose and MCC were measured by the Karl-Fischer method and was estimated to be 10.7, 14.2, 10.1, 8.3, 1.3, 1.1, 0.6, 0.1, 0.2 and 8.0%, respectively. The anhydrous forms of CDs were obtained by drying CDs in vacuum at 110 °C for 3 h and the water content of each CD was limited to be less than 1%. All other chemicals used were of reagent grade.

### Powder X-ray diffraction (PXRD) measurement

PXRD was carried out on a Rigaku Miniflex diffractometer (Rigaku Corporation, Japan). Measurements were performed at 30 kV voltage, 15 mA current, a scanning speed of 4 °/min, a Ni filter and a radiation source of Cu  $K_{\alpha}$ .

### Preparation of ground mixtures

Cyclodextrin and ONO-8713 were physically mixed at various kinds of molar ratios (CD:drug) in a glass vial by using a vortex mixer (physical mixtures, PMs). To control the moisture during the co-grinding process, a desirable amount of distilled water was added and mixed homogeneously with the PM of anhydrous CD and ONO-8713. Ground mixture (GM) was obtained by grinding the PM in a vibrational rod mill (TI-200, Heiko Seisakusho, Japan) for 10 min. The GMs with other additives, i.e., mannitol, glucose, sucrose and MCC, were prepared at 5:1 weight ratio (additive:drug).

### Determination of ONO-8713 recovered as nanoparticles

Fifty milligrams of GM was suspended in 10 ml distilled water and the suspension was sonicated for 5 min. The suspensions of GM were filtered through 0.8  $\mu$ m

membrane filter (Millipore, MA, USA). The filtrates containing nanoparticles smaller than 0.8  $\mu$ m were added into ethanol. The amounts of ONO-8713 were spectrophotometrically determined at a wavelength of 279 nm using Shimadzu UV-160 spectrophotometer. The ratio of the amount of ONO-8713 nanoparticles (<0.8  $\mu$ m) to the total amount of ONO-8713 in the suspension was calculated and expressed as the nanoparticle yield.

### Particle size analysis

The GM was dispersed in distilled water and sonicated for 2 min. The volumetric particle size distribution of each suspension was determined by the light scattering method using Microtrac FRA<sup>®</sup> (Nikkiso, Japan; Measurement range, 0.1–700  $\mu$ m) and by the dynamic light scattering method using Microtrac UPA<sup>®</sup> (Nikkiso, Japan; Measurement range, 0.003–6  $\mu$ m). Particles larger than 0.8  $\mu$ m were cut-off by membrane filter before the measurement on the UPA<sup>®</sup>.

### Zeta-potential measurement

Zeta-potential was measured for the suspensions and the filtrates of GMs by electrophoretic light scattering spectrophotometer ELS-6000 (Otsuka Electronics, Japan).

## Results and discussion

### Co-grinding with $\beta$ -CD: influence of moisture condition and $\beta$ -CD content

The intact form of ONO-8713 showed the mean particle size of 40  $\mu$ m. To perform the nanoparticle formation of ONO-8713, co-grinding between ONO-8713 and  $\beta$ -CD was carried out for 10 min using vibration mill. The grinding molar ratio ( $\beta$ -CD:ONO-8713) was varied as 1:2, 1:1, 2:1 and 5:1. We have reported previously that the moisture condition during the co-grinding process was an important factor for nanoparticle formation [13, 14]. To evaluate the influence of moisture content, the moisture during the co-grinding process was controlled to be 2–16% by adding a desirable amount of water to the PMs. PXRD analysis revealed that the GMs existed in an amorphous state when the moisture content was controlled from 2% to 12%. Characteristic X-ray diffraction peaks of both ONO-8713 and  $\beta$ -CD crystals were observed when the moisture content was more than 12% (data not shown). The changes in the PXRD pattern as a function of water content was similar to that of pranlukast system previously reported. The GM powder of ONO-8713 and  $\beta$ -CD was dispersed into distilled water so as to evaluate the nanoparticle formation. The particle size distribution of GM suspension determined by using Microtrac FRA<sup>®</sup> indicated the existence of fine particles smaller than 1  $\mu$ m. The nanoparticles in the range of 40–200 nm were observed

with the volumetric mean particle size of 120 nm after the suspension was filtered through 0.8  $\mu\text{m}$  membrane (Microtrac UPA<sup>®</sup>). Micron-sized particles in the suspension were cut off by 0.8  $\mu\text{m}$  membrane filter and the amount of ONO-8713 nanoparticles found in the filtrate was quantitatively determined. Figure 1 shows the relationship between percent yield of nanoparticles (<0.8  $\mu\text{m}$ ) and water amount in the mixtures in the  $\beta$ -CD system. Percent yield of ONO-8713 nanoparticle was increased with the increase of water amount and achieved maximum value at 12% moisture at all mixing molar ratio samples, even at 16% water content the yield diminished. Moreover, the system of higher  $\beta$ -CD content exhibited higher yield of nanoparticle formation. The maximum yield was as much as 85% in  $\beta$ -CD:drug system at a molar ratio of 5:1 and water content of 12%. As discussed previously, the interaction between  $\beta$ -CD hydrate and drug might lead to the formation of micro disordered assembly or drug nanocrystallites after grinding [7]. The CD molecules might form a network structure with neighboring CD molecules together with a solid-state interaction between drug and CD molecules. Cyclodextrin network and the suitable amount of water would help stabilize drug nanocrystallites. When the GM powder was dispersed into distilled water, the CD matrix dissolved and then drug nanocrystallites would be released and assembled together to be nanoparticles with mean particle size of 120 nm.  $\beta$ -Cyclodextrin molecules should adsorb on drug nanoparticle and form a  $\beta$ -CD layer preventing an aggregation of particles. The decrease of nanoparticle yield when moisture content exceeded 12% was ascribable to the recrystallization of ONO-8713 and  $\beta$ -CD during grinding as confirmed by PXRD analysis.

Zeta-potential was measured for the filtrated suspensions of GMs of various molar ratios prepared at 12% moisture and the results are shown in Figure 2. The intact ONO-8713 particles had a zeta potential of  $-1.6$  mV, whereas the zeta potential after co-grinding

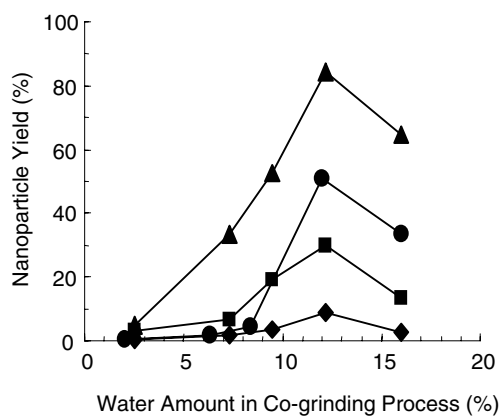


Figure 1. Effect of  $\beta$ -CD and water contents on the nanoparticle formation. Molar ratio of  $\beta$ -CD:ONO-8713 (▲) 5:1, (●) 2:1, (■) 1:1, (◆) 1:2.

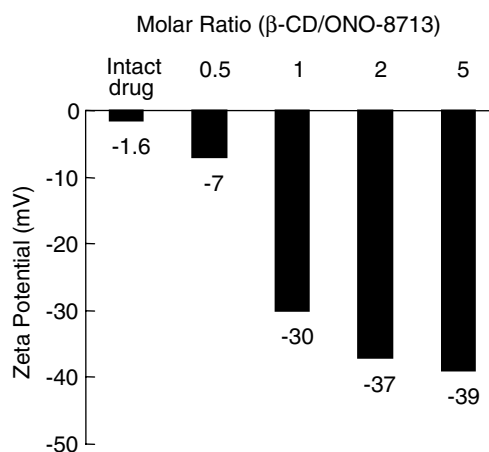


Figure 2. Zeta potential of the filtrate of GMs with different mixing molar ratio.

changed with  $\beta$ -CD content. The magnitude of zeta-potential was drastically increased when the molar ratio of  $\beta$ -CD:ONO-8713 was 1:1, which would be closely related to the nanoparticle formation in distilled water because nanoparticle yield of ONO-8713 was negligible for the molar ratio of  $\beta$ -CD:ONO-8713 was 0.5 (1:2 in Figure 1). The zeta-potential did not change when the intact particles of ONO-8713 existed in  $\beta$ -CD solution. It seems reasonable to consider that  $\beta$ -CD would adsorb on and interact with a surface of nanoparticles, altering the charge from  $-1.6$  to  $-39$  mV. The  $\beta$ -CD adsorbed layer should prevent the aggregation of particles and help stabilize nanoparticles.

#### Co-grinding with other cyclodextrins and non-cyclodextrin additives

The nanoparticle formation after dispersing GMs co-ground with various CDs (molar ratio of CD: ONO-8713 = 2:1) into distilled water are shown in Figures 3 and 4. The results in  $\alpha$ -CD and  $\gamma$ -CD systems were similar to those of  $\beta$ -CD, i.e., a sharp increase followed

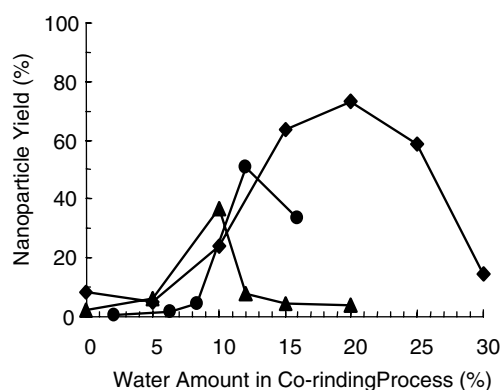


Figure 3. Percent yield of ONO-8713 nanoparticles from  $\alpha$ -,  $\beta$ - and  $\gamma$ -CD/ONO-8713 GMs as a function of water amount in co-grinding process (▲)  $\alpha$ -CD, (●)  $\beta$ -CD, (◆)  $\gamma$ -CD.

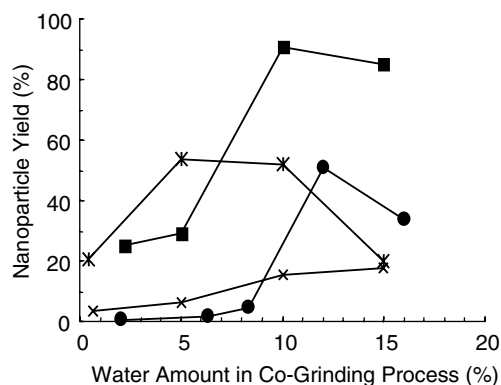


Figure 4. Percent yield of ONO-8713 nanoparticles from  $\beta$ -CD derivatives/ONO-8713 GMs as a function of water amount in co-grinding process (●)  $\beta$ -CD, (■) HP- $\beta$ -CD, (\*) DM- $\beta$ -CD, (×) TM- $\beta$ -CD.

by rapid decrease of the percent nanoparticle yield as a function of water content. The required water amount to obtain maximum yield were different in each system depending on the types of CDs. The nanoparticle yield obtained was in the superior order of  $\gamma$ -CD,  $\beta$ -CD and  $\alpha$ -CD (Figure 3).  $\gamma$ -Cyclodextrin has two hydrate forms, which are  $7\text{H}_2\text{O}$  (intermediate form) and  $17\text{H}_2\text{O}$  (stable form). We have reported that when the moisture content in the mixture was equal to the water content of CD hydrate form, the recovery value tended to increase to maximum value. As reported previously, this amount of water would help keep CD in the hydrate form which might have higher affinity to drug molecule than anhydrate form, leading to the interaction between CD and drug molecules and then consequently resulting in the nanoparticle formation [14]. The stable hydrate form of  $\gamma$ -CD ( $17\text{H}_2\text{O}$ ) contains about 19% water content. Therefore it required more moisture in the system for  $\gamma$ -CD to form  $17\text{H}_2\text{O}$  and to obtain maximum recovery. The higher recovery in  $\gamma$ -CD system than in  $\beta$ -CD and  $\alpha$ -CD is probably due to the better ability to interact with drug molecules and the larger cavity size of  $\gamma$ -CD. For  $\beta$ -CD derivatives, nanoparticle formation was in the superior order of HP- $\beta$ -CD, DM- $\beta$ -CD,  $\beta$ -CD and TM- $\beta$ -CD (Figure 4). Nanoparticle formation was hardly observed in the case of TM- $\beta$ -CD, because it has totally 21 methyl substitution groups which probably were in a position to hinder the interaction between CD and ONO-8713. Hydroxypropyl- $\beta$ -CD and  $\beta$ -CD have been widely used as hosts for the inclusion complex formation signifying that both CDs commonly interacted with drugs more easily when compared to methylated  $\beta$ -CD [16, 17]. In the previous chapter, we proposed that CD would adsorb on and interact with a surface of nanoparticle of ONO-8713. The strength of interaction between CD and ONO-8713 might affect the nanoparticle formation. The above results show that not only the amount of water in the system but also the interaction between drug and CDs was a significant factor for nanoparticle formation.

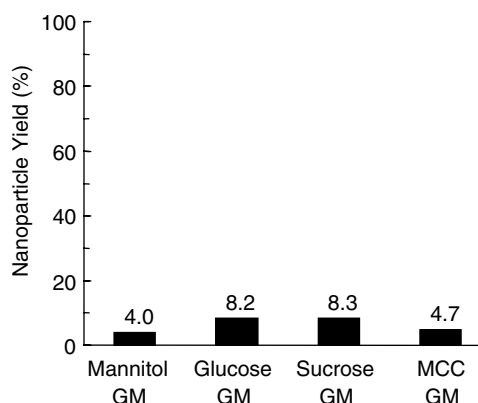


Figure 5. Percent yield of ONO-8713 nanoparticles from GMs prepared with various additives. Weight ratio of additive:ONO-8713 = 5:1, grinding time 10 min.

Figure 5 shows the nanoparticle yield when ONO-8713 was co-ground with various kinds of non-CD additives, e.g., mannitol, glucose, sucrose and MCC. The co-grinding was performed at the weight ratio of 5:1 (additive:drug) for 10 min. All GMs exhibited nanoparticle yield of less than 10%, indicating that drug nanoparticles would be hardly formed when non-CD additives were used as co-grinding additive. A major cause for the fact that nanoparticles could not be formed might be the extremely weak interaction between additives and drug or the insoluble property of additive (MCC).

## Conclusion

The method of co-grinding with CDs could be applied effectively as a nanoparticle preparation method to a poorly water soluble drug, ONO-8713. The amount of water in the system, the CD content in the mixture, and the interaction between drug and CDs were significant factors for nanoparticle formation. The difference in the cavity size of CDs along with the variation of substitution groups had influence on the interaction between CDs and drug.

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