Preparation of Drug Nanoparticles by Co-grinding with Cyclodextrin: Formation Mechanism and Factors Affecting Nanoparticle Formation

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The aim of this study was to investigate the factors affecting the formation of pranlukast nanoparticle prepared by co-grinding with β-cyclodextrin (β-CD) and to elucidate the mechanism of nanoparticle formation. The effects of grinding time, moisture content and CD content on the nanoparticle formation were evaluated by means of UV quantitative determination and particle size analysis. High-resolution scanning electron microscopy (HRSEM) was employed to observe drug nanoparticles in the ground mixture. Nanoparticle recovery was higher than 95% for 2 : 1 molecular mixtures of β-CD : pranlukast which had been ground for 10 min with moisture levels between 10 and 15%. While that of the 1 : 2 ground mixture prepared at 8% moisture level was only 57%. Nanoparticle recovery from β-CD : pranlukast 2 : 1 mixture ground for 1 min was 2.5%, while that of the 10 min ground mixture was as high as 95%. HRSEM demonstrated that primary drug nanoparticles having a particle size around 50 nm were observed in the ground mixture. The grinding time, the moisture content, and the CD content had significant influences on the formation of drug nanoparticles. The CD matrix may form and stabilize primary particles by its interaction with the particle surface through water molecules. Primary nanoparticles existed in the ground mixture as 50 nm drug nanocrystallites.

Key words nanoparticle; co-grinding; cyclodextrin; pranlukast

Several techniques have been widely applied to enhance the bioavailability of poorly water-soluble drug, for examples, inclusion complex formation,[1,2] polymeric nanoparticle,[3] solid dispersion,[4] and particle size reduction by grinding.[5,6] Micron-sized particles can be produced by grinding but particles at the nanometer level are difficult to obtain by the dry milling method. In the past few years, there are many publications focusing on the preparation of drug particles at the nanometer level by co-grinding with additives.[7—14] In previous studies, we have reported the formation of pranlukast nanoparticle with mean particle size around 200 nm by co-grinding with CDs.[10] Co-grinding with CDs could be applied as a nanoparticle preparation method for various kinds of poorly water-soluble drugs.[10—12] We also found that moisture content in the co-grinding process was an important factor and a suitable moisture condition during co-grinding was indispensable for nanoparticle formation.[13]

Pranlukast is a cysteinyl leukotriene receptor antagonist which is chemically described as (4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran). Pranlukast is practically insoluble (1.2 μg/ml H_2O at 25 °C), resulting in poor absorption after oral administration.

Environmental scanning electron microscopy (ESEM) can be used to observe samples in a moist condition by controlling water vapor pressure in the microscope specimen chamber. The observation of wet organic samples by ESEM has been reported recently.[14,15]

In this study, the effects of grinding time, moisture content and CD content on the nanoparticle formation were evaluated by means of UV quantitative determination and particle size analysis. High-resolution scanning electron microscope was employed to observe the surfaces of the ground mixtures prepared under various conditions. Moreover, ESEM was used to observe the drug particles after dispersal into water. The mechanism for nanoparticle formation was discussed.

Experimental

Materials Pranlukast hemihydrate was received from Ono Pharmaceutical Co., Ltd., Japan as jet-milled material. β-Cyclodextrin (β-CD) was kindly supplied by Nihon Shokuhin Kako Co., Ltd., Japan as a hydrate form (β-CD·10.5H_2O). The water content of β-CD was measured by the Karl–Fischer method and was found to be 14.2 w/w%. The anhydrous form of β-CD was obtained by drying β-CD·10.5H_2O in vacuum at 110 °C for 3 h and the water content was limited to less than 1%. All chemicals used were of reagent grade.

Preparation of Ground Mixtures The physical mixture of anhydrous β-CD and pranlukast was prepared at molar ratios of 1 : 2, 1 : 1, and 2 : 1 (β-CD : pranlukast) in a glass vial using a vortex mixer. To control the moisture content during the co-grinding process, the required amount of distilled water was added to the physical mixture and mixed homogeneously. The ground mixture was obtained by grinding the physical mixture in a vibrational rod mill (TI-200, Heiko Seisakusho, Japan) with a frequency of 1430 rpm and an amplitude of 8 mm for 10 min. To evaluate the effect of grinding time on nanoparticle formation, co-grinding time was varied from 1 to 120 min. Each ground mixtures were hand-ground and sieved before use and the homogeneity of the ground samples was confirmed.

Particle Size Analysis The ground mixture was suspended in distilled water (5 mg/ml) and the suspension was sonicated for 2 min. The particle size was determined by the light scattering method using a Microtrac FRA® (Nikkiso, Japan; measurement range, 0.1—700 μm). The mean volume was used as the mean particle size.

Determination of Drug Nanoparticle Recovered in the Filtrate The suspension was filtered through a 0.8 μm membrane filter (Millipore, MA, U.S.A.). Three milliliters of ethanol was added to 3 ml of the filtrate and then the mixture was diluted properly by 50% ethanol. The amount of drug was determined spectrophotometrically at a wavelength of 255 nm using a Shimadzu UV-160 spectrophotometer. The amount of drug in the filtrate relative to the total amount of drug in the suspension was calculated and expressed as percentage recovery.

Scanning Electron Microscopy Pranlukast hemihydrate, 10-min ground β-CD and the β-CD : pranlukast 2 : 1 ground mixtures were observed by high resolution scanning electron microscope (HRSEM) (Nova 200 Nanolab, FEI Company™).

Environmental Scanning Electron Microscopy A suspension of the β-CD : pranlukast 2 : 1 mixture (5 mg/ml) was poured into the sample holder

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and placed in the measurement chamber. The temperature inside the chamber was controlled to be 3 °C. To vaporize water in the suspension, the relative humidity inside the chamber was decreased from 100 to 75% by reducing pressure. Drug nanoparticles in the wet sample were observed by an environmental scanning electron microscope (ESEM) (Quanta 200, FEI Company™).

Results and Discussion

Effect of Cyclodextrin Content and Moisture Content The moisture content in the co-grinding process was a significant factor for the preparation of drug nanoparticle by co-grinding with CDs.[13] In the present study, the effect of CD content on the nanoparticle formation was investigated. β-Cyclodextrin and pranlukast were mixed at molar ratios of 1 : 2, 1 : 1 and 2 : 1 and then ground under various moisture conditions. Figure 1 shows the recovery of pranlukast obtained from the ground mixture at various molar ratios as a function of moisture content. As pranlukast and pranlukast–β-CD complex have very low aqueous solubility, the solubilization was almost negligible. Therefore the recovery of pranlukast in the filtrate is considered as drug nanoparticles. In the previous study,[13] we have reported that a suitable moisture condition was necessary for nanoparticle formation. Cyclodextrin could not interact with drug particle surface when co-grinding was performed in dry conditions, and consequently resulted in an aggregation of particles. The recrystallization of CD would occur during co-grinding in high moisture content, which also resulted in less CD–drug interaction. The maximum recovery of drug nanoparticle was almost 98% for the β-CD : pranlukast mixtures prepared at 2 : 1 molar ratio with the moisture level between 10 and 15%. The maximum nanoparticle recovery of the mixture prepared at 1 : 2 molar ratio with the moisture level of 8% was found to be only 57%. The suitable moisture amount varied depending on the amount of β-CD. As reported previously, the maximum recovery could be obtained when the moisture amount in the mixture was almost equal to the water amount in the hydrate form.[13] The required moisture amount was lower in the molar ratio 1 : 2 (β-CD : pranlukast) than in the molar ratio 1 : 1 or 2 : 1, due to a smaller amount of β-CD. When there was 10% moisture in the 1 : 2 (β-CD : pranlukast) mixture (which was equal to 18.6% calculated against only β-CD), β-CD started to recrystallize during grinding and the nanoparticle could not be formed. However, 10% moisture in the 1 : 1 mixture was equal to 14.3% moisture calculated against only β-CD, which was almost equal to the water amount in the hydrate form. Drug nanoparticles were formed and the maximum recovery was then obtained.

We have already proposed that CD may interact with the particle surface, preventing aggregation and stabilizing the nanoparticles when drug primary particles were formed on grinding. In the 1 : 1 and 2 : 1 systems (β-CD : pranlukast), the amount of CD was sufficient to stabilize drug nanoparticles. As a result, more than 95% of drug could be recovered as nanoparticles. The low recovery from the ground mixture at 1 : 2 molar ratio should be due to the incomplete matrix formation of CD.

In the systems with molar ratio of 1 : 2, 1 : 1 and 2 : 1 (β-CD : pranlukast), the weight contents of β-CD were equal to 54%, 70% and 82%, respectively. We proposed that there is a critical weight content of β-CD to form a matrix stabilizing drug nanoparticle. From the reason that 95% of drug recovered as nanoparticles at 70% β-CD weight content (molar ratio of β-CD : pranlukast = 1 : 1), whereas the maximum recovery of drug nanoparticles was only 57% at 54% β-CD weight content (molar ratio of β-CD : pranlukast = 1 : 2), the critical weight content of β-CD was apparently between 54% and 70% w/w. When β-CD was used above the critical weight content, such as 70% and 82%, drug nanoparticles were possible which were dissociated and dispersed separately in the continuous phase of CD matrix. On the contrary, at a β-CD weight content of around 50% w/w, the amount of CD was insufficient to form a continuous matrix to stabilize drug nanoparticles. As a result, drug nanoparticles could not disperse independently and the nanoparticles aggregated to form large particles. The above results indicate that not only the moisture content but also the CD content is a significant factor for the nanoparticle formation.

Effect of Grinding Time To evaluate the effect of grinding time on the nanoparticle formation, β-CD and pranlukast were mixed at a molar ratio of 2 : 1 and the moisture amount in the mixture before grinding was equal to 13.6%. The co-grinding time was varied from 1—120 min. Particle size distribution patterns of the samples are shown in Fig. 2. The physical mixture showed particle sizes in the range of 1—200 μm with a mean particle size (mean volume) of 26 μm. After co-grinding for 1 min, particles larger than 20 μm were not observed and the mean particle size was reduced to around 4 μm. The mean particle size was found to

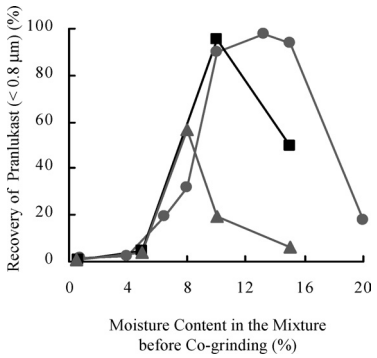

Fig. 1. The Effect of β-CD Content and Moisture Content on Nanoparticle Formation in β-CD : Pranlukast System

Molar ratio of β-CD : pranlukast, (●) 2 : 1, (■) 1 : 1, (▲) 1 : 2.

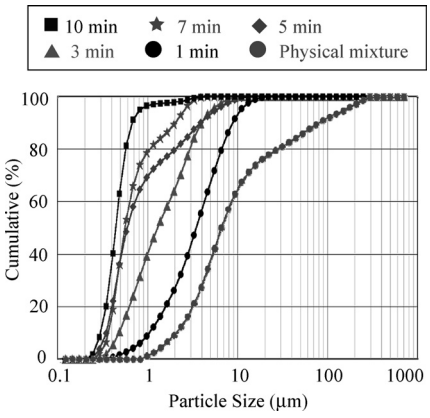

Fig. 2. Particle Size Distribution Patterns of Pranlukast Formed from β-CD : Pranlukast (2 : 1 Molar Ratio) Physical Mixture and Ground Mixtures Prepared at Various Grinding Time

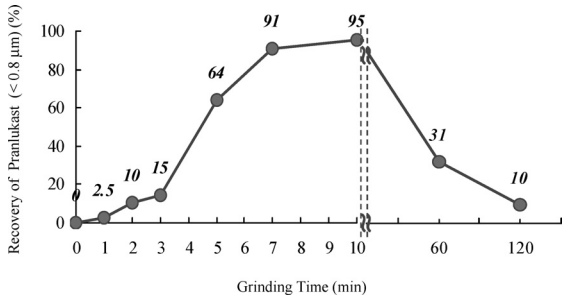

Fig. 3. The Effect of Grinding Time on the Nanoparticle Formation of β-CD : Pranlukast (2 : 1 Molar Ratio) Ground Mixture

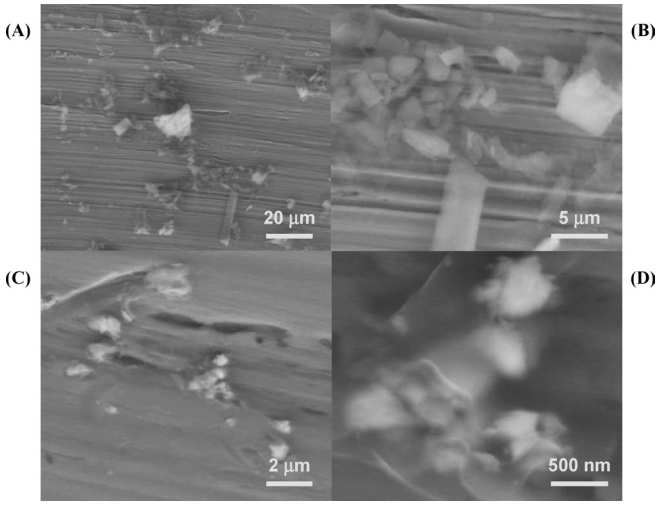

Fig. 4. ESEM Photographs of Suspensions of β-CD : Pranlukast (2 : 1 Molar Ratio) Ground Mixtures

(A) 1-min ground mixture; (B) magnification of (A); (C) 10-min ground mixture; (D) magnification of (C).

be 3.26, 1.88, 1.43 and 0.84 μm for the mixtures ground for 2, 3, 5 and 7 min. The mean particle size decreased with longer grinding time and reached the nanometer level at 7 min. The results from quantitative determination demonstrated that the nanoparticle fraction of the mixture ground for 1 min was only 2.5% while that of the mixture ground for 10 min was as high as 95% (Fig. 3). The mixtures ground for more than 10 min consisted of larger particles and exhibited lower nanoparticle fraction. It is probable that the moisture content of the mixture decreased after co-grinding for a long time and consequently resulted in the aggregation of nanoparticles.

Environmental scanning electron microscopy (ESEM) can be used to observe samples in a moist condition.[14,15] Particles in a suspension of the ground mixtures were observed by ESEM after decreasing the relative humidity in the measurement chamber by reducing pressure. Plate shaped pranlukast crystals as well as pranlukast particles in micron size, were observed in the suspension of the mixture ground for 1 min. On the other hand, particles observed in the suspension of the mixture ground for 10 min appeared to be mostly nanoparticles (Fig. 4). These results agree with the results from particle size analysis and quantitative determination.

β-Cyclodextrin was received as a hydrate form. To study the effect of grinding time, β-CD hydrate form was used to co-grind with drug. To study the effect of β-CD content and moisture content, β-CD was dried before use and the moisture was added to the mixture. The transformation from anhydrous β-CD to β-CD hydrate was observed after adding water. However, the transformation did not complete immediately but gradually occurred. Therefore, we speculated that even some water molecules existed as crystal water, some of them still remained as adsorbed water in the β-CD matrix. The water in β-CD hydrate might take part in the co-grinding as crystal water, while the water added to anhydrous CD before co-grinding could be involved as crystal water and adsorbed water. From Fig. 1, the recovery of the ground mixture prepared at 13% added moisture (molar ratio of β-CD : pranlukast = 2 : 1, ground for 10 min) was 94%. In Fig. 3, the moisture amount before co-grinding of the mixture prepared with β-CD hydrate (molar ratio of β-CD : pranlukast = 2 : 1) was 13.6% and the recovery at 10 min was 95%, which was in a good agreement with the results from Fig. 1. From the above results, we could conclude that, for β-CD, both hydrate water and added water had the same effect on the formation of nanoparticles. However, for γ-CD, the recovery of the ground mixture of indomethacin and intact γ-CD (hydrate form) showed much higher recovery than the ground mixture prepared with added moisture, implying that the position of water molecules in γ-CD might be important for nanoparticle formation.[11] This subject would although remain to be further investigation.

Surface Property of the Ground Mixtures Observed by HRSEM In the previous study, we reported that co-grinding in different moisture conditions resulted in mixtures with differing characteristics. The mixture prepared by grinding with anhydrous β-CD exhibited poor wettability and no formation of drug nanoparticles. On the other hand, the mixture prepared with β-CD hydrate show good wettability and up to 95% recovery of drug nanoparticles. In an attempt to understand the reason for these differences and to elucidate the mechanism for nanoparticle formation, observations of the sample surface were performed using high-resolution scanning electron microscope (HRSEM).

Pranlukast crystals, β-CD ground for 10 min and the β-CD : pranlukast mixtures (molar ratio = 2 : 1) were observed by HRSEM and the results are shown in Fig. 5. Pranlukast maintained a plate shape habit even after jet milling (Fig. 5A), whereas β-CD ground for 10 min exhibited a smooth surface (Fig. 5B). In the case of the mixture prepared with anhydrous β-CD, small needle-like particles are observed in the powder of the ground mixture (Fig. 5C). These particles were considered to be micronized pranlukast crystals formed by co-grinding with anhydrous β-CD. The co-grinding efficiency was low when performed under dry conditions. This was thought to be due to the fact that the molecular interaction between pranlukast and β-CD could not take place, and therefore pranlukast nanoparticles could not be produced. The micron-sized needle-like pranlukast particles seemed to aggregate rigidly and cover the whole surface of the amorphous β-CD resulting in the sample with hydrophobic surface. These could explain the poor wettability of the ground, anhydrous β-CD : pranlukast mixture.

In contrast, 50 nm pranlukast particles were observed in the mixture after co-grinding with β-CD hydrate for 10 min (Fig. 5D). These nanoparticles were dispersed homogeneously in the β-CD matrix. Co-grinding under suitable

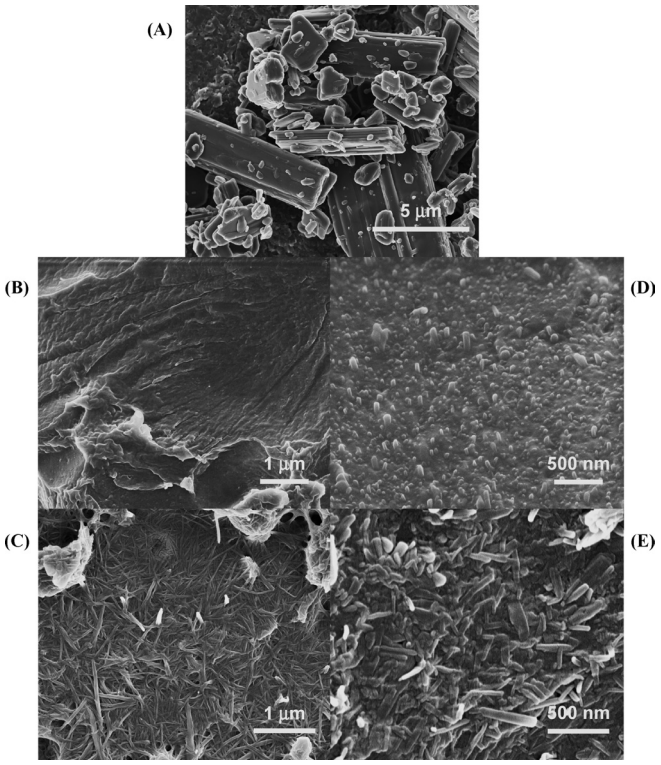

Fig. 5. SEM Photographs of Pranlukast, Ground β-CD and β-CD : Pranlukast (2 : 1, Molar Ratio) Ground Mixtures

(A) Pranlukast; (B) β-CD ground for 10 min; (C) β-CD anhydrate : pranlukast 10-min ground mixture; (D) β-CD : pranlukast 10-min ground mixture; (E) β-CD : pranlukast 1-min ground mixture.

moisture conditions resulted in better drug–CD interaction, therefore drug nanoparticle could be effectively produced. The surface of the β-CD matrix was accountable for the good wettability of the sample. The mixture with β-CD hydrate which had been ground for 1 min exhibited low nanoparticle fraction (2.5%) which is similar to the nanoparticle fraction of the anhydrous β-CD ground mixture (1.4%). However, it should be noted that these two samples showed different surfaces as shown in Figs. 5C and E. The existence of water molecules during co-grinding clearly has a great influence on the surface interaction between pranlukast and CD. For the mixture with β-CD hydrate which had been ground for 1 min, the nanoparticles had hardly formed yet. Pranlukast could interact with β-CD *via* water molecules and begin to dissociate and disperse into the β-CD matrix. The image in Fig. 5E can be considered to be the beginning stage of nanoparticle formation.

The Mechanism for Nanoparticle Formation A mechanism for nanoparticle formation is proposed as follows. When the co-grinding of drug and CD was performed with suitable moisture content, CD molecules interacted with the surface of the nano-sized drug particles through the water molecules and prevented aggregation of the drug particles. The CD matrix would form in the same time when enough CD was used. The continuous phase of the CD matrix stabilized the nanoparticles, leading to the formation of primary particles with a size around 50 nm. On the other hand, CD could not interact with drug particle surface when co-grinding was performed in dry conditions, and consequently resulted in an aggregation of particles. The recrystallization of CD would occur during co-grinding in high moisture content,[11] which resulted in less CD–drug interaction and low nanoparticle formation. The suitable water content was necessary for the establishment of drug–CD interaction as well as for the formation of the CD matrix. The surface interactions leading to nanoparticle formation seemed to be completed after 7 min of grinding. Grinding for a long time did not produce smaller particles it decreased the moisture content of the mixture and consequently resulted in the aggregation of nanoparticles. When the ground mixture was dispersed into water, the primary nanoparticles would be released after the CD matrix dissolved. These primary drug particles would agglomerate to be nanoparticles with mean particle size around 200 nm as reported previously.[10]

Conclusions

The moisture content and the CD content had significant influences on the formation of drug nanoparticles. The existence of water molecules during the co-grinding clearly had a great influence on the surface interaction between CD and drug. The interaction between CD and drug particle surface through the water molecules occurred during co-grinding with suitable moisture resulting in the stabilization of primary drug particles. Co-grinding in dry or high moisture condition resulted in less drug–CD interaction and lower nanoparticle formation. The surface interaction leading to the nanoparticle formation seemed to be completed after 7 min of grinding. The moisture content of the mixture decreased after co-grinding for a long time and consequently resulted in the aggregation of nanoparticles.

The differences between the sample surfaces of the anhydrous β-CD ground mixture and the mixture with β-CD hydrate ground for 1 min were observed by high-resolution scanning electron microscopy. When pranlukast and β-CD were ground in a suitable condition, the primary drug particles would form and dissociated in the CD matrix. These primary particles would agglomerate to form drug nanocrystallites with a mean particle size around 200 nm when the ground mixture was dispersed into water.

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