Journal of Inclusion Phenomena and Macrocyclic Chemistry (2006) 56: 29–32
DOI 10.1007/s10847-006-9056-7

Investigation of Drug Nanoparticle Formation by Co-grinding with Cyclodextrins: Studies for Indomethacin, Furosemide and Naproxen

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Key words: co-grinding, cyclodextrin, indomethacin, nanoparticle, naproxen, poorly water-soluble drug, solubilization

Abstract

Drugs with poor water solubility were co-ground with cyclodextrins (CDs) to create nanoparticles with improved solubility characteristics. Indomethacin (IDM), furosemide (FRM) and naproxen (NAP) were co-ground with β-CD at the molar ratio of 2:1 (CD:drug). Co-grinding of a drug with CD resulted in not only the formation of drug nanoparticles but also the solubilization of the drug by inclusion complex formation with CD in aqueous media. The nanoparticle fraction of IDM, and FRM from ground mixtures prepared with β-CD was as high as 60–70% while the solubilization fraction was less than 10%. In contrast, β-CD–NAP ground mixture showed a large fraction, 48%, for drug solubilization and only 4% for nanoparticle formation. Furosemide ground mixtures prepared with α-CD, β-CD and γ-CD showed comparatively high nanoparticle fraction while the solubilization fraction was around 10%. Both the nanoparticle fraction and the solubilization fraction were greater in the IDM–β-CD system than those in γ-CD and α-CD systems. The nanoparticle formation of NAP depended on the types of CD used as a co-grinding additive. Naproxen nanoparticles could be prepared by co-grinding NAP and α-CD, while the solubilization of NAP tended to improve when β-CD or γ-CD was used.

Introduction

Oral administration of drugs with low aqueous solubility usually results in poor absorption and bioavailability. One of the techniques that has been widely applied for drug bioavailability enhancement is the size reduction method [1, 2]. Micron-sized particles can be obtained by grinding method, however, particles at nanometer level are difficult to produce by dry milling. Recently, co-grinding of drug with some additives, e.g., cyclodextrin (CDs) [3–5], D-mannitol [6] or some water-soluble polymers [7], was found to be a simple and effective method to prepare drug nanoparticles. Previously, we reported the formation of crystalline nanoparticles by co-grinding with various kinds of CDs [3]. We also found that moisture content in the co-grinding process was a significant factor and a suitable moisture condition was indispensable for nanoparticle formation [4]. Pranlukast hemihydrate and ONO-8713 nanoparticles, having mean particle size around 192 and 120 nm, were successfully prepared with high nanoparticle yields by using this method [5]. The nanoparticles also exhibited good physicochemical stability.

In this study, we apply the method of co-grinding with CDs to other poorly water-soluble drugs. Drug nanoparticle formation was investigated using indomethacin (IDM), furosemide (FRM), and naproxen (NAP).

Experimental

Materials

Indomethacin, FRM and NAP were purchased from Wako Pure Chemical Industries, Ltd., Japan and were used without further purification. α-Cyclodextrin and β-cyclodextrin were kindly supplied from Nihon Shokuhin Kako Co. Ltd., Japan. γ-Cyclodextrin was purchased from Junsei Chemical Co. Ltd., Japan. Water contents of intact α-CD, β-CD and γ-CD were determined by the Karl–Fischer method and were estimated to be 10.0, 14.0 and 9.5%, respectively. Anhydrous forms of the CDs were obtained by drying CDs in vacuum at 110 °C for 3 h and the water content of each

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CD was limited to less than 1%. All other chemicals used were of reagent grade.

Preparation of ground mixtures

Cyclodextrin and the drug were physically mixed at molar ratio of 1:1, 1.5:1, and 2:1 in a glass vial by using a vortex mixer (physical mixtures). To control the moisture during the co-grinding process, the required amount of distilled water was added and mixed homogeneously with the physical mixture. The ground mixture was obtained by grinding the physical mixture in a vibrational rod mill (CMT TI-200, Japan) for 30 min.

Determination of drug recovered in the filtrate

Fifty mg of the ground mixture was suspended in 10 ml of distilled water and the suspension was sonicated for 3 min. The suspension was filtered through an 11 μm pre-filter followed by a 0.8 μm membrane filter. Three milliliter of ethanol was added to 3 ml of the filtrate and then the mixture was adequately diluted. The amount of drug was determined spectrophotometrically using 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. After 1 ml of the filtrate was centrifuged at 15,000×*g* for 10 min, the amount of drug in the supernatant was determined spectrophotometrically and expressed as percentage solubilization. The percentage of nanoparticle formation was calculated from the difference of recovery and solubilization.

Particle size analysis

The ground mixture was dispersed in distilled water and sonicated for 3 min. The volumetric particle size distribution of each suspension was determined by dynamic light scattering method using Microtrac UPA® (Nikkiso, Japan; Measurement range, 0.003–6 μm). Due to the measurement limitation of Microtrac UPA® of 6 μm, the suspension was passed through a 5 μm membrane filter before measurement.

Results and discussion

In the previous study, we reported pranlukast nano order particle formation in the aqueous suspension of the ground mixture of pranlukast and β-CD. As pranlukast and pranlukast–β-CD complex have very low aqueous solubility, the solubilization was almost negligible. Hydrophobic organic compounds with functional groups suitable for inclusion complex formation are solubilized in β-CD aqueous solution as a result of inclusion compound formation. We proposed that a solubilization of a drug might occur simultaneously with the nanoparticle formation after dispersion of the ground mixture into aqueous media. To evaluate the mechanism of nanoparticle formation, the percentage recovery was determined from the sum of the percentage of nanoparticle formation and the percentage of solubilization. β-Cyclodextrin hydrate (β-CD·10H_2O) was used to co-grind with IDM, FRM and NAP at the molar ratio 2:1 (β-CD:Drug) for 30 min. The nanoparticle fraction and solubilization fraction of each drug are shown in Figure 1. The results from quantitative determination indicated that co-grinding of these drugs and β-CD resulted in not only the formation of drug nanoparticles but also the solubilization of the drug by inclusion complex formation with CD. The nanoparticle formation of FRM and IDM was as high as 70 and 62%, respectively, while the solubilized drug amounts were less than 10%. In contrast, NAP showed 48% drug solubilization and only 4.0% nanoparticle formation. The difference would depend on the ability (solubility of the complex and stability constant) of drug to form inclusion complex with CDs.

Indomethacin, FRM and NAP were further investigated by using α-CD and γ-CD as co-grinding additives. Figure 2 shows the percentage nanoparticle formation and the percentage solubilization of IDM from the ground mixtures prepared with α-CD, β-CD and γ-CD at 1:1, 1.5:1 and 2:1 molar ratios. In the β-CD system, the nanoparticle fraction reached 60% for all molar ratios but it was lower when α-CD and γ-CD were used. Both the nanoparticle fraction and the solubilization fraction were found to be greater with β-CD than with γ-CD or α-CD. In addition, the nanoparticle formation of IDM tended to increase with increasing CD amount. The influence of moisture content on IDM nanoparticle formation was investigated for α-CD, β-CD and γ-CD systems and the results are shown in Figure 3. The formation of drug nanoparticles clearly depends on the moisture condition. The nanoparticle fraction is less than 5% for ground mixtures of α-CD or β-CD

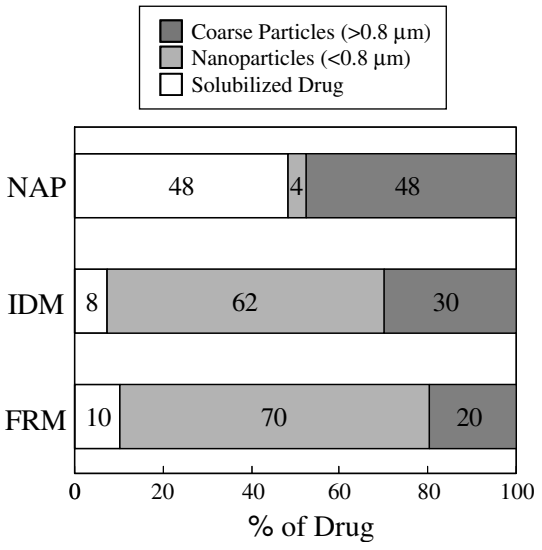

Figure 1. Percentage of solubilized amount, nanoparticles and coarse particles in aqueous suspensions of β-CD:drug ground mixtures (molar ratio β-CD:drug = 2:1).

prepared at 0–7.5% and 0–5% moisture levels, respectively. In γ-CD, the ground mixtures prepared at 10% water content exhibited only 4.0% nanoparticle formation. Nevertheless, the maximum percentage of nanoparticle fraction (around 60%) was obtained for ground mixtures prepared with α-CD, β-CD and γ-CD at 10, 15 and 12–15% water content, respectively. The observed changes in percent of nanoparticles as a function of moisture content is similar to that of pranlukast reported previously, although the patterns of change vary according to the kind of drug and the types of CDs. These results confirm the necessity of suitable water content in co-grinding for nanoparticle formation. In the cases of ground mixtures prepared with intact α-CD and β-CD (molar ratio CD:drug = 2:1), the moisture content before co-grinding was 8.4 and 12.1%. The nanoparticle fractions of the ground mixture (Figure 1) agree with the results in Figure 3. It should be noted that the nanoparticle fraction of the ground mixture prepared with intact γ-CD was much higher than those prepared at 10% moisture. Even the moisture content in the mixture of IDM and intact γ-CD before co-grinding was only 8.3%. γ-CD contains 17 molecules of crystal water at a relative humidity (RH) of 93.6%. When the RH is between 30 and 50%, the intact γ-CD is known to be in an intermediate form, containing seven water molecules, between the anhydrate form and the $17H_2O$ form [8]. The water in the intact γ-CD might take part in the co-grinding as crystal water, while the water added to anhydrous γ-CD before co-grinding could be involved as adsorbed water. These results imply that the position of water molecules in γ-CD might be important for nanoparticle formation, although this subject remains to be investigated further.

The solubilization and nanoparticle formation of NAP were investigated using α-CD, β-CD and γ-CD (Figure 4). Among three ground mixtures (molar ratio of CD:NAP = 2:1), the one with β-CD exhibited the highest solubilization fraction of 48% and a very low nanoparticle fraction of only 4.0%. When α-CD was used, the solubilization fraction decreased to around 10% whereas the nanoparticle fraction increased to 37%. On the other hand, the ground mixture with γ-CD exhibited only the solubilization of drug, indicating no nanoparticle formation. The results of the ground mixtures at molar ratio of 1:1 and 1.5:1 show a similar

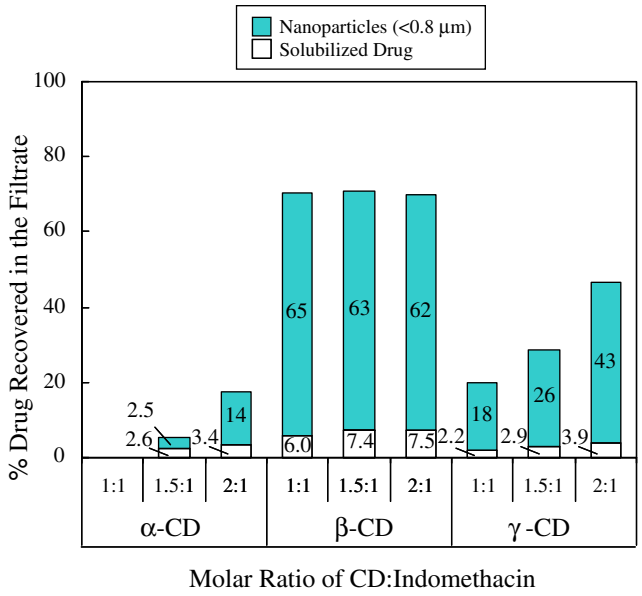

Figure 2. Percentage of solubilized amount and nanoparticles in aqueous suspensions of CD:IDM ground mixtures at various molar ratios.

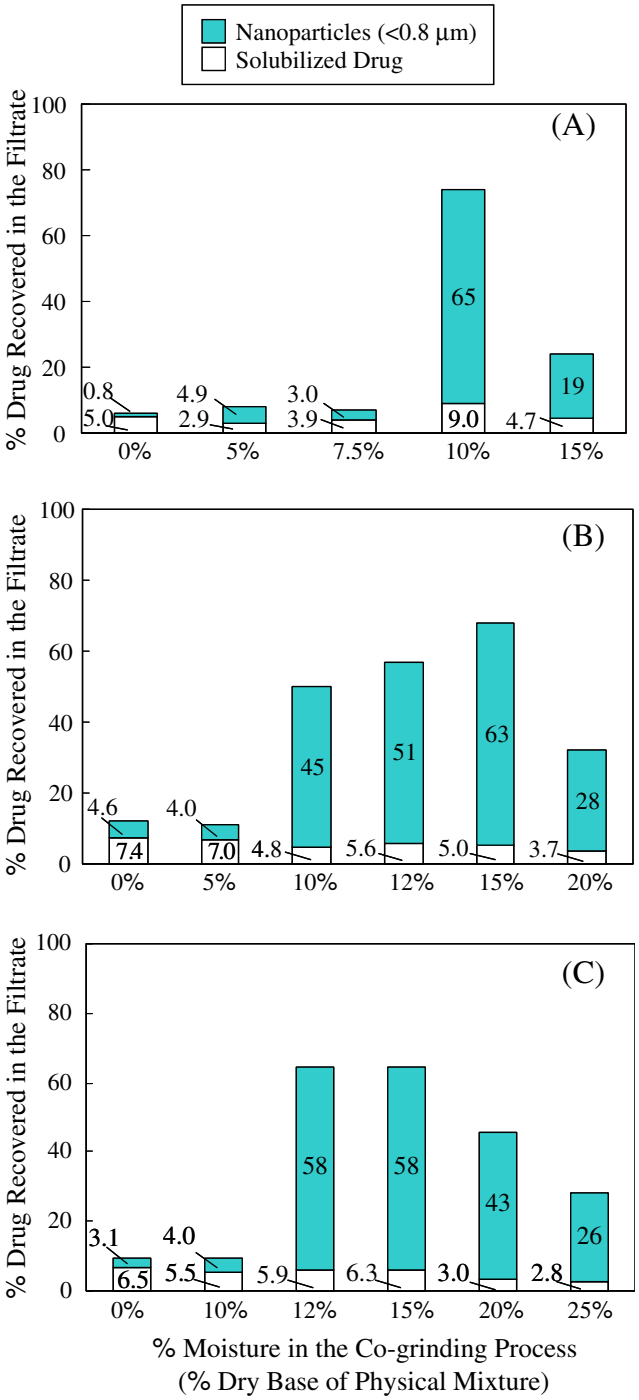

Figure 3. Influence of moisture content in the co-grinding on IDM nanoparticle formation in (A) α-CD, (B) β-CD and (C) γ-CD systems (molar ratio CD:IDM = 2:1).

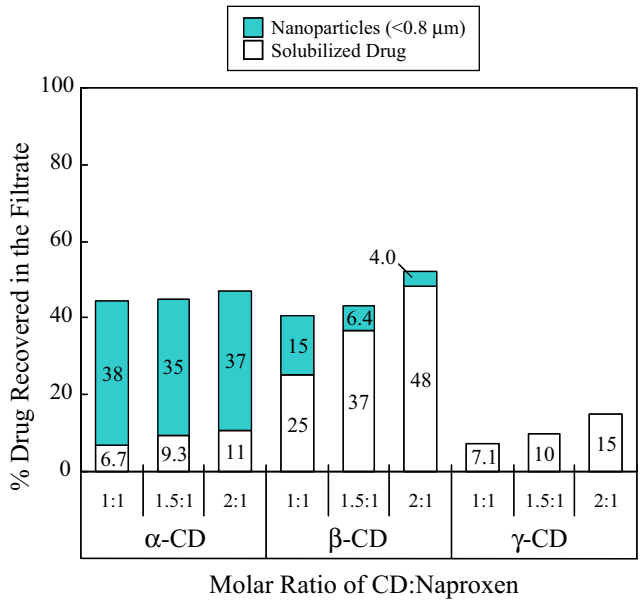

Figure 4. Percentage of solubilized amount and nanoparticles in aqueous suspensions of CD:NAP ground mixtures at various molar ratios.

feature to those of the 2:1 ground mixtures. Naproxen nanoparticles were prepared with comparatively high yield by the co-grinding of NAP and α-CD, while the solubilization of NAP tended to improve when either β-CD or γ-CD was used. In the case of NAP, co-grinding with a large cavity CD resulted in inclusion complex formation, and hence less nanoparticle formation. As in the γ-CD–NAP system, NAP can easily form an inclusion complex with γ-CD and therefore the formation of nanoparticles was not observed. According to Faucci *et al*. [9], the stability constants of NAP–CD complexes at molar ratio of 1:1 in water at 25 °C were found to be 40, 1700 and 146 M^{-1} for α-CD, β-CD and γ-CD, respectively. Based on NMR and computer molecular graphic studies, Bettinetti *et al*. reported that NAP fits well into the cavity of β-CD and therefore the inclusion complex had better stability than those with α-CD or γ-CD [10]. This could explain why the solubilization of β-CD–NAP ground mixture was higher than that of γ-CD.

Conclusions

Co-grinding of some poorly water-soluble drugs with CDs results in the formation of nanoparticles together with the solubilization of the drug. The types and cavity sizes of the CD, the moisture content in the co-grinding system, as well as the ability of drug to form inclusion complex with CD, significantly affects the formation of nanoparticles and the solubilization of the drug.

Acknowledgments

This study was supported by Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology (Monbukagakusho) of Japan. We thank Nihon Shokuhin Kako Co. Ltd., for the kind gift of α-CD and β-CD.

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