Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 265 (2003) 85–93
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Formation of fine drug particle by cogrinding with cyclodextrins Part II. The influence of moisture condition during cogrinding process on fine particle formation

Arpansiree Wongmekiat [a], Yuichi Tozuka [a], Toshio Oguchi [b], Keiji Yamamoto [a,*]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*
[b] *Department of Pharmacy, University Hospital, University of Yamanashi, 1110, Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi 409-3898, Japan*

Received 25 April 2003; received in revised form 23 July 2003; accepted 25 July 2003

Abstract

The purpose of this study was to investigate the effect of moisture condition during cogrinding process on fine drug particle formation. Cogrinding of cyclodextrins (CDs) and pranlukast (PRK) hemihydrate was performed in various moisture conditions at a mixing molar ratio of 2:1 (CDs:PRK) and the formation of PRK submicron particle was investigated. The moisture content in the cogrinding process significantly affected the fine particle formation. More than 90% of pranlukast loaded transformed to submicron particles when coground with α-CD, β-CD or γ-CD containing the specific amount of water for each CD system. Fine particle formation of PRK was considered as a particular phenomenon to cyclodextrins, since the submicron particles could not be formed when D-mannitol, lactose or microcrystalline cellulose (MCC) was used as a cogrinding additive. Moreover, the appearance and disappearance of fine particle formation was found to be reversible depending on the existence of water during the grinding process.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Submicron particle; Cyclodextrin; Pranlukast; Cogrinding; Poorly water-soluble drug

1. Introduction

The enhancement of aqueous solubility of poorly water-soluble drugs is one of the most essential topics in the pharmaceutical area. Several techniques have been applied in attempt to improve drug solubility. Microemulsion technique (Itoh et al., 2002), solid dispersion (Verheyen et al., 2002), and complexation (Dollo et al., 1999; Trapani et al., 2000) have been widely used for the solubility enhancement. Size reduction method has also been employed extensively (Buckton and Beezer, 1992; Martini et al., 1991) and the important advantage of this method is the increase in the surface area which may enhance dissolution rate and consequently bioavailability of pharmaceutical materials. Size reduction of pharmaceutical materials is often performed by means of dry milling process, but the limitation of size reduction by dry milling is known to be around 3 μm due to the aggregation between particles. Recently, the researches focusing on particle size reduction to submicron region by cogrinding with some additives have been attempted (Kubo et al., 1997; Liversidge and Cundy, 1995; Sugimoto et al., 1998; Yamada et al., 1999). The method of size re-

* Corresponding author. Tel.: +81-43-290-2937; fax: +81-43-290-2939.
E-mail address: yamamotk@p.chiba-u.ac.jp (K. Yamamoto).

0378-5173/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0378-5173(03)00409-5

duction is promising but still has some difficulties in application.

Pranlukast (PRK) hemihydrate (4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4*H*-1-benzopyran·1/2H_2O) is a cysteinyl leukotriene receptor antagonist which is used as an anti-asthmatic agent. PRK exhibits extremely low aqueous solubility (1.2 μg/ml H_2O at 25 °C) resulting in poor absorption when administered orally and low bioavailability.

In the previous paper, we reported a formation of PRK fine particle by cogrinding with β-CD·10.5H_2O (Wongmekiat et al., 2002), where 96% of loaded PRK transformed to fine particles in submicron level (mean particle size: 192 nm) when the β-CD·10.5H_2O/PRK ground mixture (GM) was dispersed in water. On the other hand, PRK fine particles could not be produced when β-CD anhydrate was used as a cogrinding additive. These results suggested that the moisture during the cogrinding process affected the formation of fine particles.

In the present study, the amount of water during the cogrinding process was systematically controlled and the effect of moisture on the formation of fine particles was evaluated in detail. The investigation was also performed in α-CD and γ-CD systems. To investigate the mechanism of fine particle formation, D-mannitol, lactose and microcrystalline cellulose were used as cogrinding additives as well. Moreover, further grinding in a different moisture condition was performed to β-CD anhydrate/PRK GM and β-CD·10.5H_2O/PRK GM.

2. Materials and methods

2.1. Materials

Pranlukast hemihydrate was received as a gift from Ono Pharmaceutical Co. Ltd., Japan. β-Cyclodextrin (β-CD) was supplied by Nihon Shokuhin Kako Co. Ltd., Japan. α-CD and γ-CD were purchased from Mercian Corporation, Japan. The anhydrous form of CDs was 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%. D-Mannitol and lactose were obtained from Nacalai Tesque, Inc., Japan. Microcrystalline cellulose (MCC) was supplied from Asahi Chemical Industry Co. Ltd., Japan. All other chemicals used were of reagent grade.

2.2. Preparation of ground mixtures

CDs and PRK were physically mixed at 2:1 molar ratio (CD:PRK) in a glass vial by using a vortex mixer (physical mixtures, PMs).

To control the moisture during the cogrinding process, a desirable amount of distilled water was added and mixed homogeneously to the PM of anhydrous CD and PRK. GM was obtained by grinding the mixture in a vibration mill (CMT TI-200) for 10 min.

The GMs with other additives, i.e. D-mannitol, lactose, and MCC, were performed at 5:1 weight ratio (additive:PRK).

2.3. Powder X-ray diffraction (PXRD) measurement

Powder X-ray diffraction was carried out on a Rigaku Miniflex diffractometer (Tokyo, Japan). Measurements were performed at 30 kV voltage, 15 mA current, a scanning speed of 4°/min with a Cu Kα radiation source.

2.4. Determination of PRK recovered as fine particles

One hundred milligram of GM was suspended in 10 ml distilled water and the suspension was sonicated for 2 min. The suspensions of GM were filtered through 0.8 μm membrane filter (Millipore, Bedford, MA, USA). The filtrates containing fine particles smaller than 0.8 μm were dissolved with ethanol. The amounts of PRK were spectrophotometrically determined at a wavelength of 255 nm using Shimadzu UV-160 spectrophotometer. The recovery value, the total yield of smaller particles than 0.8 μm, was calculated according to the Eq. (1):

$$\text{Recovery (\%)} = \frac{\text{Amount of PRK fine particles } (< 0.8\,\mu\text{m})}{\text{Total amount of PRK in the suspension}} \times 100 \quad (1)$$

2.5. Particle size analysis

The GM was dispersed in water and sonicated for 2 min and the suspension was passed through 0.8 μm

membrane filter (Millipore) before the measurement. Particle size was determined by the dynamic light scattering method using Microtrac UPA® (Nikkiso, Japan; measurement range, 0.003–6 μm).

2.6. Humidification

Humidification of GMs was performed by storage of samples in a desiccator containing saturated aqueous solution of KCl for 82% relative humidity (RH) at 40 °C for 5 months. The water content of the GMs after humidification was determined to be 11.5% by Karl Fischer method.

2.7. Further grinding of β-CD/PRK GMs at the different moisture conditions

The β-CD·10.5H_2O/PRK GM was dried at 110 °C for 3 h, and then the dried sample was further ground in a vibration mill for 10 min. On the other hand, distilled water was added to the dried sample of β-CD anhydrate/PRK GM to obtain the sample of 13% water amount and the further grinding was performed.

3. Results and discussions

3.1. Influence of the moisture condition during cogrinding process

The moisture content during the cogrinding process was systematically controlled to clarify the effect of water amount on ultrafine particle formation. The amount of water was adjusted by adding a desirable amount of water to the mixture of β-CD anhydrate and PRK before cogrinding. Then, PRK fine particle formation from the GMs was evaluated. The amount of PRK found in the filtrate which passed through 0.8 μm membrane filter was quantitatively determined. The amount of PRK particles in the filtrate to the total PRK amount in the suspension was expressed as the recovery value. Fig. 1 shows the relationship between recovery value and water amount in the mixtures in β-CD system (molar ratio β-CD:PRK = 2:1), where water amount during cogrinding process was controlled from 0.75% (β-CD anhydrate GM) to 20%. The results showed an immediate sharp increase of the recovery value when the water amount was in the range of 4–10%. Almost all of PRK transformed to fine particles when the water amount was 13%, while the amount of fine particles decreased rapidly when the moisture exceeded 13%. At 20% moisture, the recovery was found to be as low as 18%.

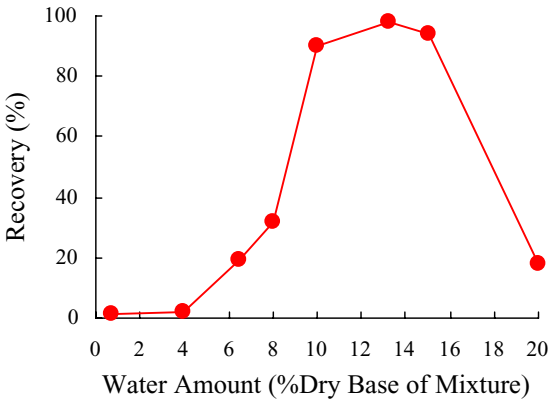

Fig. 1. Fine particle fraction in the suspensions of β-CD/PRK GMs as a function of water amount in GMs (molar ratio; β-CD: PRK = 2:1, ground for 10 min).

Fig. 2 shows the XRD patterns of PRK, β-CD, PM and GMs. PRK crystals exhibited sharp XRD peaks at $2\theta = 3.3, 9.9, 14.4, 16.6$ and 19.9°. The GM coground with β-CD anhydrate, the GMs containing 4.0, 6.5 and 8.0% moisture showed the halo XRD patterns (Fig. 2d–g), suggesting the amorphization of PRK and β-CD. However, the XRD peak of PRK at $2\theta = 3.3^\circ$ and small peaks due to β-CD crystals were observed in the patterns of the GMs containing in 13 and 15% moisture (Fig. 2i and j). When the water amount in the mixture was increased to 20%, the XRD peaks of β-CD hydrate crystals were observed (Fig. 2k), showing the recrystallization of β-CD by grinding in high moisture condition.

α-CD and γ-CD were also used as cogrinding additives (molar ratio CD:PRK = 2:1). The relationship between the recovery values and water amounts in the mixtures are shown in Fig. 3. The results of α-CD and γ-CD system showed the similar manner to that of β-CD, i.e. the sharp increase of the recovery value followed by rapid decrease of the recovery value, whereas the maximum recovery values were obtained at 8.0 and 20 moisture in α-CD and γ-CD system, respectively. The XRD peak of PRK at $2\theta = 3.3^\circ$ and small peaks of CDs were observed in the XRD patterns of

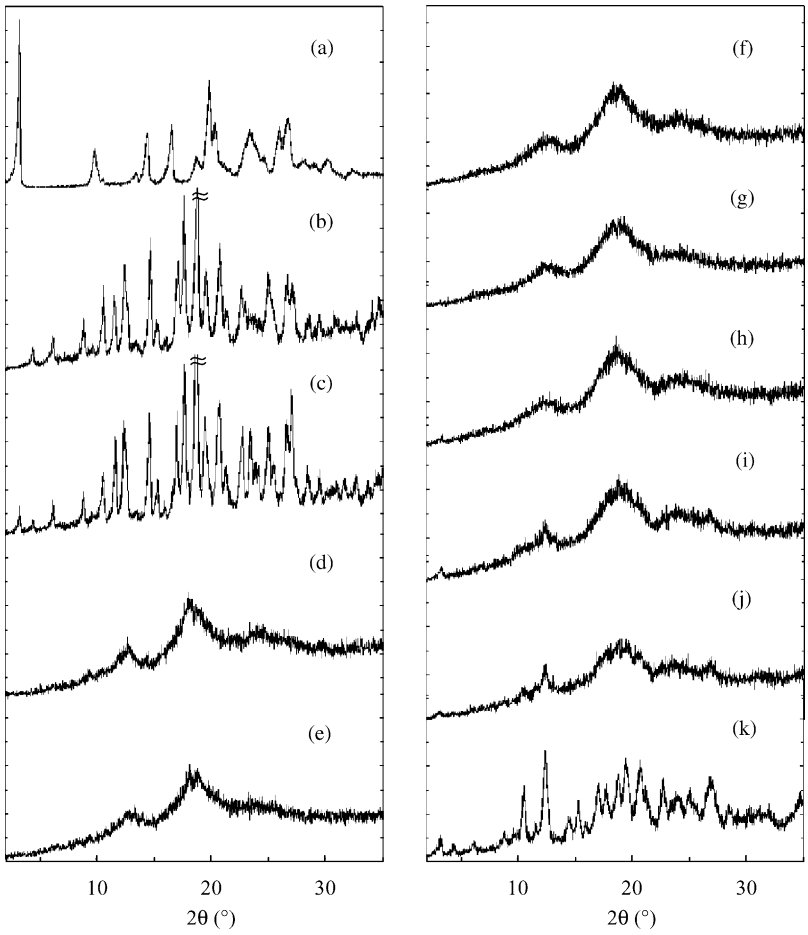

Fig. 2. PXRD patterns of β-CD/PRK GMs (a) intact PRK, (b) β-CD·10.5H_2O, (c) physical mixture, (d) GM with 0.75% water amount (β-CD anhydrate GM), (e) GM with 4.0% water amount, (f) GM with 6.5% water amount, (g) GM with 8.0% water amount, (h) GM with 10% water amount, (i) GM with 13% water amount, (j) GM with 15% water amount, (k) GM with 20% water amount (molar ratio; β-CD:PRK = 2:1, ground for 10 min).

the GMs which exhibited the highest recovery values. Grinding GM in a higher moisture condition promoted the recrystallization of α-CD hydrate and the appearance of γ-CD hydrate crystals resulted in low recovery value. The required water amount to obtain maximum recovery values which were different in each system indicated that there was an optimum range of moisture for fine particle formation, and that moisture played an important role for fine particle formation.

From the above results, we speculated that water molecules not only acted as a lubricant at the molecular level during the cogrinding process, but also as a binder for drug and CD molecules and that CD and PRK caused a partial inclusion at the surface. The partial inclusion phenomenon between the CD hydrate and PRK might lead to the formation of submicron particles. Then, CD molecules and the suitable amount of water would help stabilize the submicron particles possibly by forming CD network covering PRK particle to prevent an aggregation between particles.

In higher moisture condition, the affinities between CD molecules increased during the grinding process, resulting in recrystallization of CDs, lower affinities between β-CD and PRK molecules, decreased cogrinding efficiency and consequently no fine particle formation.

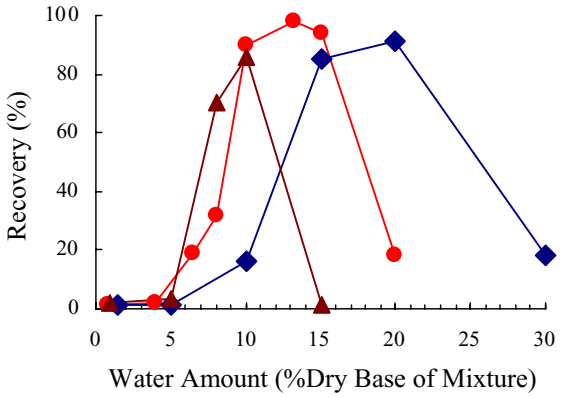

Fig. 3. Fine particle fraction in the suspensions of CD/PRK GMs as a function of water amount in GMs (molar ratio; CD:PRK = 2:1, ground for 10 min) (▲) α-CD, (●) β-CD, (◆) γ-CD.

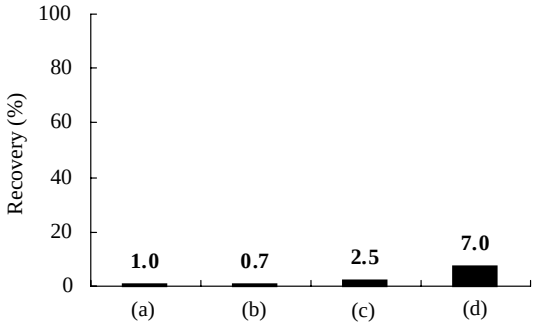

Fig. 4. Fine particle fraction in the suspensions of mannitol/PRK GMs. Weight ratio of mannitol:PRK, H_2O%, grinding time: (a) 5:1, 0.67%, 10 min; (b) 5:1, 5.0%, 10 min; (c) 5:1, 0.67%, 60 min; (d) 9:1, 0.73%, 10 min.

It has been reported that α-CD, β-CD and γ-CD had maximum hydrate forms of $6H_2O$, $12H_2O$ and $17H_2O$, respectively, and it should be noted that a great amount of fine particles could be obtained when the amount of water in the mixture was almost equal to the water amount in each hydrate form. This result suggested that the hydrate forms of CDs might play an important role in the fine particle formation.

We assumed that the CD hydrate form had much superior affinity to PRK molecules than the anhydrate form. This reflected in the difference of the degree of drug particle formation.

3.2. Cogrinding with other additives

To investigate the mechanism of PRK fine particle formation, D-mannitol, lactose and MCC were used as cogrinding additives. The cogrinding was performed at 5:1 weight ratio (additive:PRK) for 10 min. After cogrinding, the GM with MCC was amorphous, however, the GMs with D-mannitol and lactose were not amorphous. The XRD peaks of the additives were clearly still observed after grinding. The three GMs exhibited very low recovery below 4%, demonstrating no fine particle formation when D-mannitol, lactose or MCC were used.

The further investigation was carried out in the D-mannitol system to evaluate the effect of moisture condition on the fine particle formation, 5.0% of water was added to the mixture and then the cogrinding was carried out. The recovery value, however, was still below 1.0%, indicating that the moisture during cogrinding process had no influence on the PRK fine particle formation in D-mannitol system (Fig. 4b). The effects of grinding time and D-mannitol content on the fine particle formation were investigated as well. When the grinding time was extended to 60 min or the content of D-mannitol was increased from 5:1 to 9:1 weight ratio (D-mannitol:PRK), the recovery of these two GMs still remained below 10%. Therefore, it can be concluded that there were no significant formation of submicron particles in D-mannitol system even though the conditions during cogrinding process, i.e. moisture, grinding time and content of additives were altered.

From the above results, fine drug particle formation can be considered as a particular phenomenon to CDs, since the submicron particles could not be formed when D-mannitol, lactose or MCC were used as cogrinding additives.

3.3. Changes of β-CD/PRK GMs after humidification

By controlling water content after cogrinding process by storing β-CD/PRK GMs at 40 °C and 82% RH, the role of H_2O molecules in fine particle formation was investigated. The recovery values of the GMs before and after the humidification are shown in Fig. 5.

For the GM prepared with β-CD anhydrate and the GM coground with 4.0% moisture, the recovery values slightly increased after the humidification but still remained below 10%. Even after the long-term storage at conditions of high humidity (5 months), the

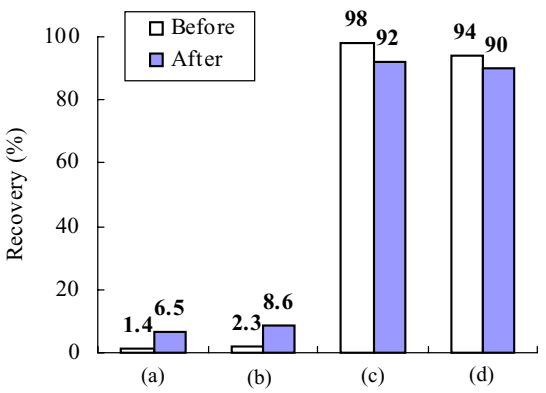

Fig. 5. Comparison of recovery values for various β-CD/PRK 2:1 10 min GMs before and after humidification (storage at 40 °C, 82% RH) (a) β-CD anhydrate GM, (b) GM with 4.0% water amount, (c) GM with 13% water amount, (d) GM with 15% water amount.

GMs with 13 and 15% water amount still showed high recovery value more than 90%. The results indicated that although the moisture during cogrinding process significantly affect the formation of drug particle, the moisture condition after cogrinding process had no significant influence on drug particle formation.

Fig. 6 shows XRD patterns of the GMs before and after the humidification. The XRD peaks of β-CD hydrate were observed after the humidification, indicating the recrystallization of β-CD. The XRD peak of PRK at 3.3° could be observed in all GMs after the humidification. For the GM coground with 13 and 15% water amount, the PRK peak intensity after the humidification was higher than that before humidification.

3.4. Further grinding of β-CD/PRK GMs at different moisture conditions

The further grinding at different moisture conditions was performed for the β-CD·10.5H_2O/PRK GM and

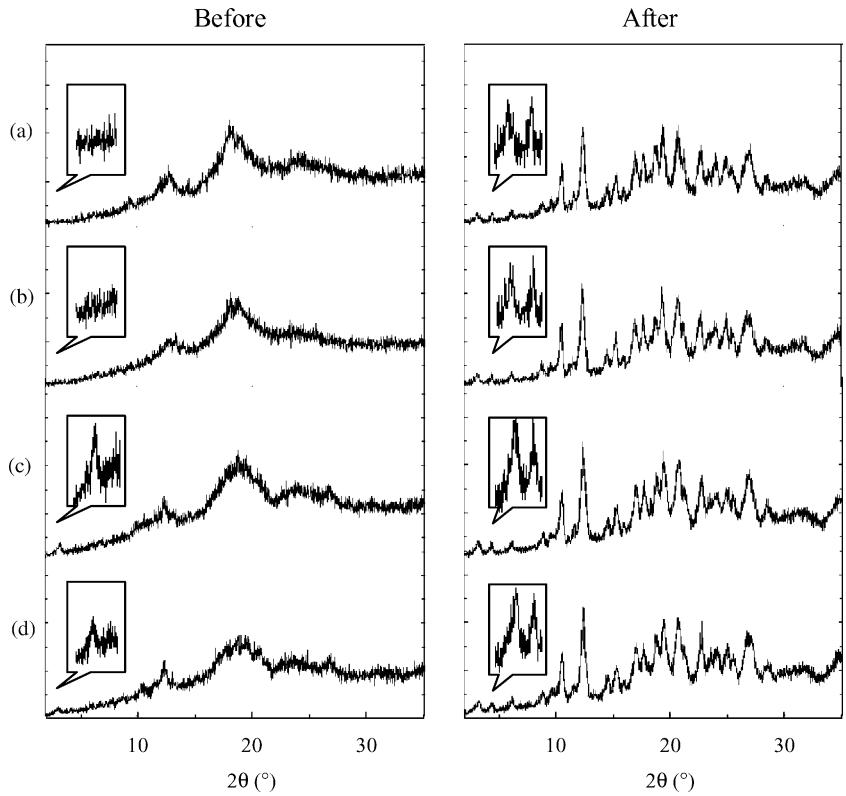

Fig. 6. PXRD patterns of various β-CD/PRK 2:1 10 min GMs before and after humidification (storage at 40 °C, 82% RH) (a) β-CD anhydrate GM, (b) GM with 4.0% water amount, (c) GM with 13% water amount, (d) GM with 15% water amount.

Table 1
Changes in PRK fine particle fraction (% recovery) after various treatment

	β-CD anhydrate/PRK GM (%)	β-CD·10.5H_2O/PRK GM (%)
(a) GM after cogrinding for 10 min	1.4	96
(b) After drying at 110 °C for 3 h of samples (a)	4.0	95
After further grinding in high moisture condition (13%) of sample (b)	84	–
After further grinding of sample (b) for 10 min	–	7.0

the β-CD anhydrate/PRK GM. At first, the effect of the removal of water from GMs was examined by drying GMs at 110 °C for 3 h. As shown in Table 1, no change in the fine particle formation was observed after drying at 110 °C for 3 h. Next, the appearance and disappearance of fine particles by cogrinding were investigated by regrinding of GMs after the drastic change of the water amount. When the dried sample of β-CD anhydrate/PRK GM which showed no fine particle formation was further ground with 13% distilled water, the drastic increase of the recovery was observed. On the other hand, significant fine particle formation was not observed when the β-CD·10.5H_2O/PRK GM was further ground in dry condition. It can be concluded that the presence and absence of drug particle formation were reversible depending on the existence of water during the grinding process. To produce fine particles, water was necessary only during the cogrinding process. Once the fine particles were formed, water had no significant influence as the drying or humidification had no effect on the recovery of drug particles.

3.5. The speculation for fine particle formation mechanism

During the grinding process, particle size reduction occurred continuously along with the aggregation of particles, nevertheless the particle size could not be reduced to submicron level in the final point. In this study, fine drug particles could be produced by cogrinding the drug with CDs in a suitable moisture condition. The possible mechanism of fine particle formation is illustrated in Fig. 7.

When drug was coground with CDs in a suitable moisture condition, the water molecules would act as a lubricant at the molecular level and therefore improve

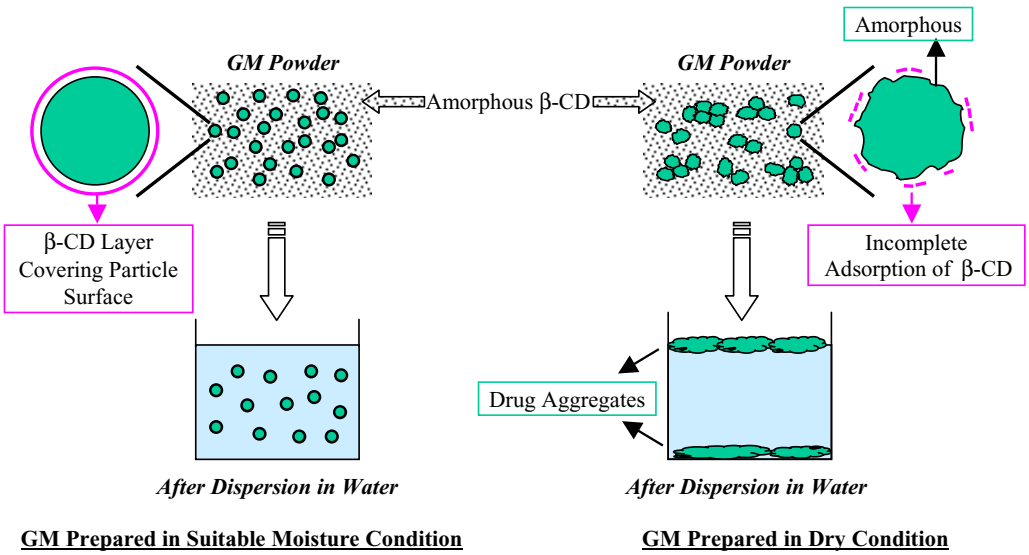

Fig. 7. The illustration of possible mechanism for fine particle formation.

grinding efficiency. When PRK was ground until the particle size reaching nanometer region, CD molecules would be bound to PRK particles causing interaction at the particle surface. The CD molecules formed a network structure with neighboring CD molecules covering the particle surface together with a solid surface interaction between PRK and CD molecules. When the GM powder which was consisted of PRK crystallites in an amorphous matrix of CD was dispersed into the water, the CD matrix dissolved and then fine PRK crystallites would be released and suspended stably in the water without aggregation. The suitable amount of water seemed to be necessary for the establishment of surface interaction processes and CD network formation. The suitable amount of water was able to keep CD molecules in the hydrate form, which had superior affinity to PRK particle surface than the anhydrate form. To produce PRK fine particles, it was found that water is necessary only during the cogrinding process. Once the interaction between CD and PRK occurred and the fine particles were stabilized in CD matrix, water molecules had no longer significant role. However, this stable structure could be destroyed if GM was further ground in dry condition. The CD network and the interaction between CD and PRK were destroyed by mechanical strength from grinding and the stable structure could not be formed again if there is no suitable water amount during the grinding. The fine particles without CD layer would begin to aggregate to be large particles.

On the other hand, when PRK was coground with CD in the dry condition, it seemed likely that the network structure and molecular interactions did not take place even particle size of PRK slightly decreased. CD existed in the anhydrate form which might have low affinity to PRK. Without suitable amount of water, CD could not be bound to and interact with the PRK particle surface, resulting in an incomplete adsorption of CD and consequently an aggregation of drug particles both in GM powder and in water.

4. Conclusion

PRK particles in submicron level could be easily produced by means of cogrinding with CDs. We found that the moisture condition during the cogrinding process significantly affected drug fine particle formation. The strong relationship between the amount of water and the amount of PRK fine particle was observed. The fine particle formation was a reversible procedure depending on the presence of water molecules during the grinding process.

Fine drug particle formation was considered as a particular phenomenon to CDs. The partial inclusion phenomenon between the CD hydrate and PRK might lead to the fine particle formation. The suitable water amount and CD molecules would help stabilize the submicron particles possibly by forming CD network covering PRK particle to prevent an aggregation between particles. When dispersing the GM in water, CD matrix would dissolve in water and then submicron size of PRK particles would be released and suspended stably in the medium.

Acknowledgements

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

References

Buckton, G., Beezer, A.E., 1992. The relationship between particle size and solubility. Int. J. Pharm. 82, R7–R10.

Dollo, G., Corre, P.L., Chollet, M., Chevanne, F., Bertault, M., Burgot, J.-L., Verge, R.L., 1999. Improvement in solubility and dissolution rate of 1,2-dithiole-3-thiones upon complexation with β-cyclodextrin and its hydroxypropyl and sulfobutyl ether-7 derivatives. J. Pharm. Sci. 88, 889–895.

Itoh, K., Matsui, S., Tozuka, Y., Oguchi, T., Yamamoto, K., 2002. Improvement of physicochemical properties of N-4472. Part II. Characterization of N-4472 microemulsion and enhanced oral absorption. Int. J. Pharm. 246, 75–83.

Kubo, H., Osawa, T., Takashima, K., Mizobe, M., 1997. Enhancement of oral bioavailability and pharmacological effect of by micronization in co-ground mixture with D-mannitol. Biol. Pharm. Bull. 19, 741–747.

Liversidge, G.G., Cundy, K.C., 1995. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: absolute oral bioavailability of nanocrystalline danazol in beagle dogs. Int. J. Pharm. 125, 91–97.

Martini, A., Torricelli, C., Ponti, R.D., 1991. Physico-pharmaceutical characteristics of steroid/crosslinked polyvinylpyrrolidone coground systems. Int. J. Pharm. 75, 141–146.

Sugimoto, M., Okagaki, T.S., Narisawa, S., Koida, Y., Nakajima, K., 1998. Improvement of dissolution characteristics and bioavailability of poorly water-soluble drugs by novel cogrinding method using water-soluble polymer. Int. J. Pharm. 160, 11–19.

Trapani, G., Latrofa, A., Franco, M., Pantaleo, M.R., Sanna, E., Massa, F., Tuveri, F., Liso, F., 2000. Complexation of zolpidem with 2-hydroxypropyl-β-, methyl-β-, and 2-hydroxypropyl-γ-cyclodextrin: effect on aqueous solubility, dissolution rate, and ataxic activity in rat. J. Pharm. Sci. 89, 1443–1451.

Verheyen, S., Blaton, N., Kinget, R., Mooter, G.V., 2002. Mechanism of increased dissolution of diazepam and temazepam from polyethylene glycol 6000 solid dispersions. Int. J. Pharm. 249, 45–58.

Wongmekiat, A., Tozuka, Y., Oguchi, T., Yamamoto, K., 2002. Formation of fine drug particles by cogrinding with cyclodextrin. I. The use of β-cyclodextrin anhydrate and hydrate. Pharm. Res. 19, 1869–1874.

Yamada, T., Saito, N., Imai, T., Otagiri, M., 1999. Effect of grinding with hydroxypropyl cellulose on the dissolution and particle size of a poorly water-soluble drug. Chem. Pharm. Bull. 47, 1311–1313.