Formation of Fine Drug Particles by Cogrinding with Cyclodextrins. I. The Use of β-Cyclodextrin Anhydrate and Hydrate

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Purpose. To improve the micromeritical properties of pranlukast (PRK) hydrate, a cogrinding process with cyclodextrin was used, and the formation of fine drug particles was investigated.

Methods. PRK crystals were ground with either β-cyclodextrin (β-CD) anhydrate or β-CD hydrate crystals at a mixing molar ratio of 2:1 (β-CD:PRK) to prepare the ground mixtures (GMs). Powder X-ray diffraction measurement and particle size analysis were performed.

Results. The two GMs differed from one another in appearance, wettability, and fine particle production. Quantitative determination demonstrated that when the β-CD hydrate/PRK GM was dispersed in water, 96% of PRK loaded in GM became fine particles smaller than 0.8 μm. In contrast, only 1.4% of PRK in GM transformed to fine particles in the case of β-CD anhydrate/PRK GM. The PRK fine particles were considered to be dispersed as small crystals. The stability of PRK particles in the aqueous solution was improved by the addition of a water-soluble polymer.

Conclusion. Cogrinding with a β-CD of higher water content can be an effective method to prepare fine drug particles at the submicron level.

KEY WORDS: pranlukast; cyclodextrin; cogrinding; micronization; fine particle; poorly water-soluble drugs.

INTRODUCTION

PRK hemihydrate (4-oxo-8-[4-(4-phenylbutoxy)benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran $1/2H_2O$) (Fig. 1) is a leukotriene antagonist that is used in the treatment of asthma. This compound has a complicated structure with five aromatic rings and exhibits very low aqueous solubility (1.2 μg/mL H_2O at 25°C), resulting in poor absorption when administered orally.

Solid dispersion (1–3), complexation (4–9), and micronization (10,11) have been applied in an attempt to improve the solubility of poorly water-soluble drugs. Micronization of pharmaceutical materials is often performed by the means of a dry milling process. However, the limitation of size reduction is known to be around 3 μm because of the aggregation between particles. The dry milling process is also used for the preparation of solid dispersion and complexation (12,13). Cogrinding drugs with some additives, such as cholic acid and cyclodextrins, is often used as a method for complexation (13,14).

Cyclodextrins (CDs) and their derivatives are widely used as host compounds. CDs have a toroidal shape, and the inner surface of their cavity is relatively hydrophobic and the outer surface hydrophilic. Because of this structure, CDs can form an inclusion complex with drug molecules and alter some physical and chemical properties of drugs (15–20). One of the most important applications of CDs is enhancing the aqueous solubility of drugs. There are numerous researchers who have reported about complex formation between CDs and poorly water-soluble drugs to improve their aqueous solubility (5–9).

Recently, experiments focusing on particle-size reduction to the submicron level by cogrinding with some additives have been attempted. Little is known of the details, although the methods of size reduction have been continuously developed (21–26). In the present study, the cogrinding process with CD was used to achieve the size reduction of pranlukast (PRK). The properties of the ground mixtures were investigated in both solid and aqueous phases. Furthermore, the particle size reduction occurring during the cogrinding process was a subject of interest.

MATERIALS AND METHODS

Materials

PRK hydrate was received as a gift from Ono Pharmaceutical Co. Ltd., Japan. β-Cyclodextrin (β-CD) was supplied by Nihon Shokuhin Kako Co. Ltd., Japan as a hydrate form (β-CD·$10.5H_2O$). The anhydrous form of β-CD was obtained by drying β-CD·$10.5H_2O$ in vacuum at 110°C for 3 h. The water content of β-CD·$10.5H_2O$ and β-CD anhydrate was measured by Karl-Fischer method and was found to be 14.2 and 1.2%, respectively. All other chemicals used were of reagent grade.

Preparation of Ground Mixtures

Cyclodextrin and PRK were physically mixed at 1:2, 1:1, or 2:1 molar ratio (β-CD:PRK) in a glass vial by using a vortex mixer (physical mixtures, PMs). For the preparation of ground mixtures (GMs), the PM of β-CD and PRK was ground in a vibration mill (CMT TI-200) for 10 min.

Powder X-Ray Diffraction (PXRD) Measurement

PXRD was performed on a Rigaku Miniflex diffractometer (Tokyo, Japan). Measurements were performed at a 30-kV voltage, 15-mA current, a scanning speed of 4° min^{-1}, and a radiation source of CuKα.

Particle Size Analysis

The GM was dispersed in water and sonicated for 2 min. Particle size was determined by the light-scattering method using Microtrac FRA® (Nikkiso, Japan; measurement range, 0.1–700 μm) and by the dynamic light-scattering method using Microtrac UPA® (Nikkiso, Japan; Measurement range,

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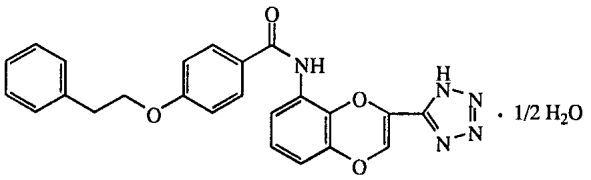

Fig. 1. Chemical structure of PRK.

0.003–6 μm). Particles larger than 0.8 μm were cut off by membrane filter before the measurement on the UPA®.

Determination of PRK Recovered as Fine Particles

The suspensions of GM (100 mg/10 mL of water) were filtered through an 0.8-μm membrane filter (Millipore, Bedford, MA, USA). The filtrates containing fine particles of 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 was calculated according to Eq. (1):

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

Estimation of Physicochemical Property for Suspended Particles

The suspension of β-CD·10.5H_2O/PRK 2:1 GM was passed through an 0.8-μm membrane filter, and the particles suspended in the filtrate were collected by using a 0.1-μm membrane filter (Millipore). PXRD was immediately performed for the residue on the 0.1-μm membrane filter. As a reference, the same procedure was used for aqueous suspension of unprocessed PRK crystals.

Physicochemical Stability Test

β-CD·10.5H_2O/PRK 2:1 GM was suspended in distilled water, 0.1% w/v hydroxypropylmethylcellulose (HPMC 2910) aqueous solution, and 0.1% w/v polyvinylpyrrolidone (PVP-25) aqueous solution. The filtrates passed through the 0.8-μm membrane filter were incubated at 30°C and measured for particle size distribution at definite intervals.

Scanning Electron Microscopy (SEM)

The suspension of β-CD·10.5H_2O/PRK 2:1 GM was passed through the 0.8-μm membrane filter and the fine particles in the filtrate were collected by using an 0.2-μm membrane filter (Millipore, Japan). PRK particles on the 0.2-μm membrane filter was observed by SEM.

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

Cogrinding of PRK with β-CD

The intact PRK was crystalline powder, which exhibits poor wettability and low aqueous solubility. To improve the solubility of PRK, cogrinding between β-CD and PRK was performed at 1:2, 1:1, and 2:1 molar ratios. Two kinds of β-CD of differing water content, β-CD anhydrate and β-CD·10.5H_2O, were used as additives. After cogrinding, the

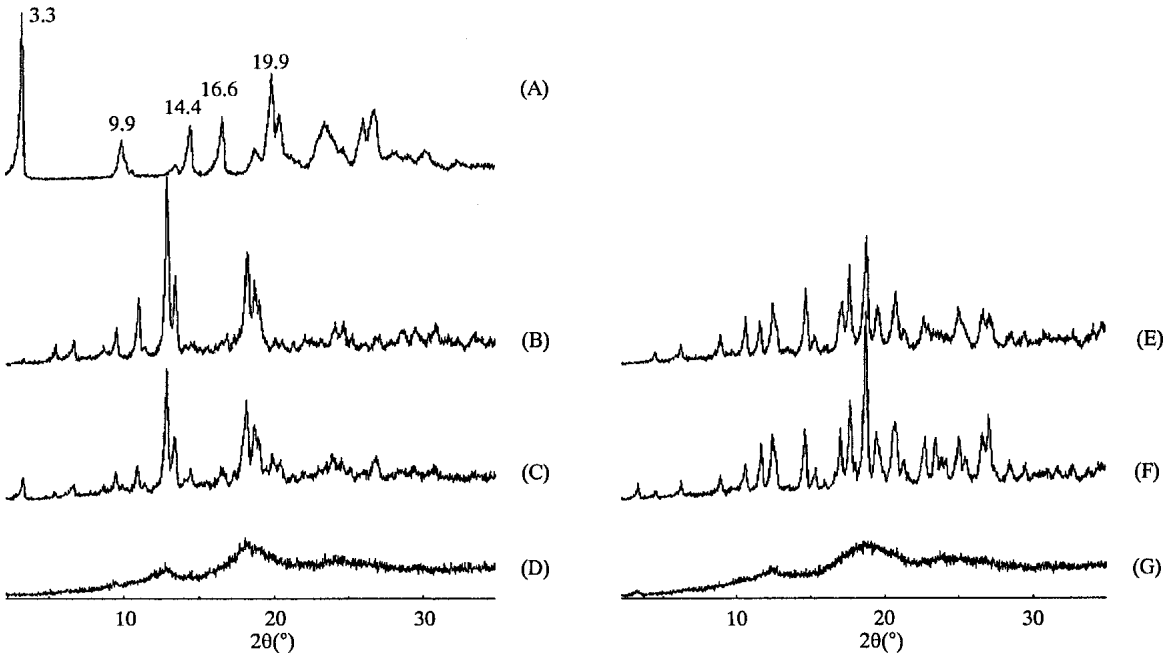

Fig. 2. PXRD patterns of β-CD/PRK Systems (molar ratio β-CD:PRK = 2:1, ground for 10 min). (A) Intact PRK; (B) β-CD anhydrate; (C) β-CD anhydrate PM; (D) β-CD anhydrate GM; (E) β-CD·10.5H_2O; (F) β-CD·10.5H_2O PM; and (G) β-CD·10.5H_2O GM.

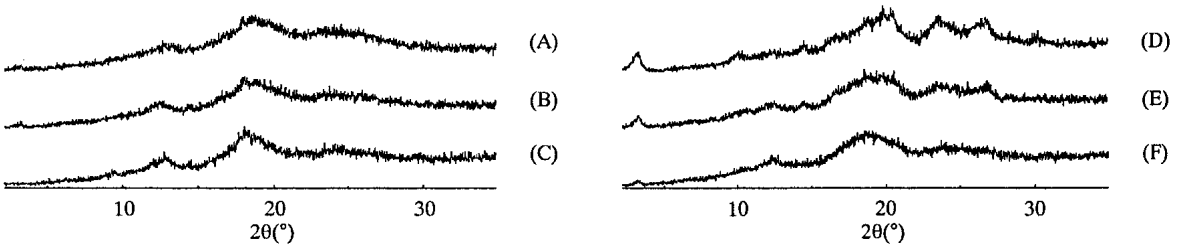

Fig. 3. PXRD Patterns of β-CD/PRK systems in various molar ratios (molar ratio β-CD:PRK, ground for 10 min). (A) β-CD anhydrate 1:2 GM; (B) β-CD anhydrate 1:1 GM; (C) β-CD anhydrate 2:1 GM; (D) β-CD·10.5H_2O 1:2 GM; (E) β-CD·10.5H_2O 1:1 GM; and (F) β-CD·10.5H_2O 2:1 GM.

powder specimens of the GMs exhibited quite different appearance. The β-CD·10.5H_2O/PRK GM became a stiff mass and clung to the grinding cell. After harvesting, the GM was pulverized with mortar and pestle. However, β-CD anhydrate/PRK GM presented as a fine powder and could be easily harvested.

Figure 2 shows PXRD patterns of PRK, β-CD, the PMs, and the GMs at 2:1 molar ratio (β-CD:PRK). PRK crystals exhibited sharp XRD peaks at 2θ = 3.3, 9.9, 14.4, 16.6, and 19.9°. On the PXRD pattern of PMs, diffraction peaks because of PRK were clearly observed as small peaks and overlapped with PXRD peaks of β-CD·10.5H_2O. Although cogrinding with β-CD anhydrate made the PXRD patterns of GM like a halo, suggesting the amorphization of PRK and β-CD, the peak of PRK crystals at 2θ = 3.3° was still observed in the PXRD pattern of β-CD·10.5H_2O/PRK GM. This indicated that PRK crystals remained to some extent, even after cogrinding for 10 min when β-CD·10.5H_2O was used. The similar results were also obtained for the GMs at 1:1 and 1:2 molar ratios, as shown in Fig. 3.

Fine-Particle Formation of PRK in the Suspension

In conventional assessment of drug solubility, solid specimens are dispersed in a medium. After equilibrated, insoluble fraction is removed by filtration with a membrane filter (pore size 0.2–0.8 μm), and then the drug concentration in the filtrate is determined by appropriate method, such as ultraviolet determination or high-performance liquid chromatography. According to the conventional method, we attempted to determine PRK solubility using β-CD·10.5H_2O/PRK GM. When the GM was dispersed in water, what was most striking was that the wettability and the aqueous dispersibility were quite improved (Fig. 4). The suspensions were filtrated through the 0.8-μm membrane filter after incubating the suspension at 30°C; however, the filtrate of the β-CD·10.5H_2O/PRK GM suspension were still turbid. The turbidity of the filtrate was found to decrease when the 0.2-μm membrane filter was used instead of the 0.8-μm membrane filter. These results suggested that there were very fine particles in submicron size in the filtrate.

Particle size analysis was performed for the suspensions and filtrates of the intact PRK, the β-CD anhydrate/PRK GM, and the β-CD·10.5H_2O/PRK GM. The dispersion of intact PRK crystals showed double-peaked distribution in particle size, in the 0.2–20 μm and 50–700 μm regions (Fig. 5A). Because the primary particle was estimated as around 10 μm by particle size analysis in dry process, the particles in the larger region were considered as a result of aggregation between PRK particles and the particles in the smaller region represented well-dispersed particles.

With regard to the β-CD·10.5H_2O/PRK GM, the particle size distribution indicated the existence of very small particles, as illustrated in Fig. 5C. After sonication, the suspension was found to consist of only fine particles smaller than 1 μm. It should be noted that the fine particles were mostly produced even by the process of just dispersing the β-CD·10.5H_2O/PRK GM in water. The measurement on the UPA was performed to the filtrates to determine a more precise particle size distribution in submicron region. The size distribution pattern exhibited a peak at 0.04–0.8 μm, and the mean particle size was 0.19 μm (Fig. 5D).

On the other hand, the β-CD anhydrate/PRK GM exhibited poor wettability, and aggregates were found floating on the water surface when dispersed in water. Figure 5B

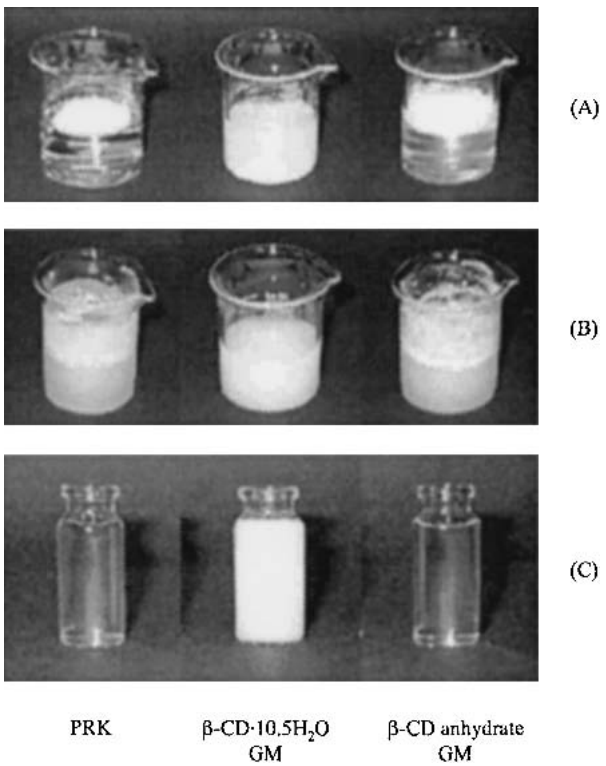

Fig. 4. Appearance of suspensions and filtrates of intact PRK and GMs (molar ratio β-CD:PRK = 2:1, ground for 10 min) before sonication (A), after sonication (B), and after filtration through an 0.8-μm filter (C).

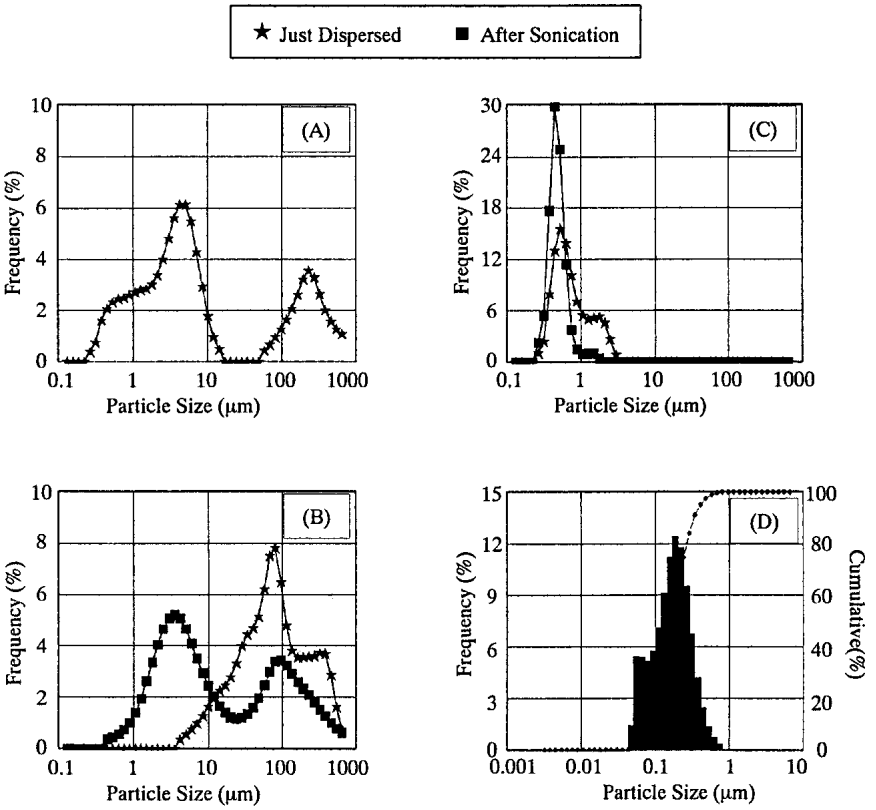

Fig. 5. Particle size distribution curves of suspensions and filtrates. (A) Suspension of PRK; (B) β-CD anhydrate GM suspension; (C) β-CD·10.5H_2O GM suspension; and (D) filtrate of (C) (passed through an 0.8-μm filter).

shows the particle size distribution pattern of the β-CD anhydrate/PRK GM. Before sonication, the suspension showed the particle size in the range of 4–650 μm. Sonication effectively changed the particle size distribution profile, and the fraction of smaller particles (1–10 μm) was found, likely the result of the deaggregation of particles. Because the filtrate of the β-CD anhydrate/PRK GM was slightly turbid after filtration with the 0.8-μm membrane filter, β-CD anhydrate/PRK GM could also produce fine particles to some extent. The UPA data confirmed the existence of fine particles in 0.04- to 0.7-μm size in the filtrate.

PRK fine particle formation from the GM was quantitatively evaluated. Table I shows the amount of PRK found in the filtrate after it was filtered through an 0.8-μm membrane filter. The PRK fraction found in the filtrate to the PRK amount in the suspension is expressed as the recovery value. When β-CD·10.5H_2O/PRK GM was dispersed in water, what interesting is as much as 96% of PRK was found to transform to fine particles. On the other hand, less than 1.4% of PRK transformed to fine particles when β-CD anhydrate/PRK GM was dispersed in water. Dispersibility of particles is affected by the zeta potential of the suspension. Zeta potentials of the suspensions of β-CD·10.5H_2O/PRK GM and β-CD anhydrate/PRK GM were found to be −60.8 mV and −37.8 mV, respectively. These results supported better dispersibility of β-CD·10.5H_2O/PRK GM in comparison with β-CD anhydrate/PRK GM.

The recovery value of β-CD·10.5H_2O/PRK GMs at other molar ratio are shown in Table II. The results showed that a great amount of PRK fine particles could be effectively produced even when the amount of β-CD·10.5H_2O used was reduced from 2:1 to 1:1 (β-CD:PRK) mixing ratio. However, the recovery value decreased to only 47% when the amount of β-CD·10.5H_2O used was reduced to 1:2 (β-CD:PRK) mixing ratio.

Table I. PRK Fine Particle (<0.8 μm) Generated from the GMs with β-CD

	Concentration of PPK particles (mg/mL)	Recovery (%)[a]
β-CD · 10.5H_2O/PRK 2:1 GM	1.52	96
β-CD anhydrate/PRK 2:1 GM	0.022	1.4
Intact PRK	0.0032	0.064

[a] Recovery = amount of PRK particles/total PRK amount in suspension; total PRK amount in suspension is 1.6 mg/mL.

Physicochemical Properties of PRK Fine Particles

When β-CD·10.5H_2O/PRK GM was dispersed in water, most of PRK was transformed to fine particles that were sus-

Table II. PRK Fine Particle (<0.8 μm) Generated from β-CD · 10.5H_2O/PRK GM in Various Molar Ratios

Molar ratio (β-CD:PRK)	Recovery (%)
2:1	96
1:1	86
1:2	47

pended in the medium. These particles might be dispersed as coacervate or crystallite form or otherwise particles could consist of a complex of CD and PRK. Thus, it is essential to investigate the properties of fine particles and how particles suspended in the aqueous solution.

The suspension of β-CD·10.5H_2O/PRK 2:1 GM was passed through an 0.8-μm membrane filter, and then the particles suspended in the filtrate were collected on 0.1-μm membrane filter. PXRD was performed on the particles on the membrane filter; the results are shown in Fig. 6. The fine particles exhibited sharp PXRD peaks at 2θ = 9.9, 14.4, 16.6, and 19.9°. Because this PXRD pattern was identical to that of PRK crystals, it was concluded that the PRK particles dispersing in water were crystallite of PRK.

A system consisting of very small particles is assumed not to be thermodynamically stable, and particle size tends to increase by crystal growth of primary particles or by aggregation between particles. To estimate the physicochemical stability of the PRK fine particles in aqueous solution, the filtrates of β-CD·10.5H_2O/PRK GM suspensions were incubated at 30°C and analyzed for size distribution patterns at definite intervals.

When distilled water was used as a dispersing medium (Fig. 7A), the filtrate immediately after filtration showed particle size distribution between 0.04–0.8 μm, and the mean particle size was 0.19 μm. After incubating for 3 days, the size distribution pattern shifted to a larger region, and the mean particle size increased from 0.19 μm to 0.48 μm. The increase in particle size was considered a result of the flocculation or aggregation between fine particles. This consideration could be supported from the SEM photograph, as shown in Fig. 8.

In the case of the aqueous polymer solutions (Fig. 7, B and C), all the patterns exhibited particle size distribution in the range of 0.04–0.8 μm. At first, the particles around 0.04–0.06 μm were observed, and the curves gradually shifted to larger region as the incubation time increased. This result suggested that the primary particles obtained after the dispersion of GM in the aqueous solution had a particle size around 0.04–0.06 μm. The particle size then gradually increased with the elongation of incubation time, but no particle larger than 0.8 μm was observed. As shown in Fig. 9, in the case of HPMC and PVP aqueous solutions, the mean particle size did not change even after 3 days of incubation. It has been reported that the addition of polymer provided highly stable

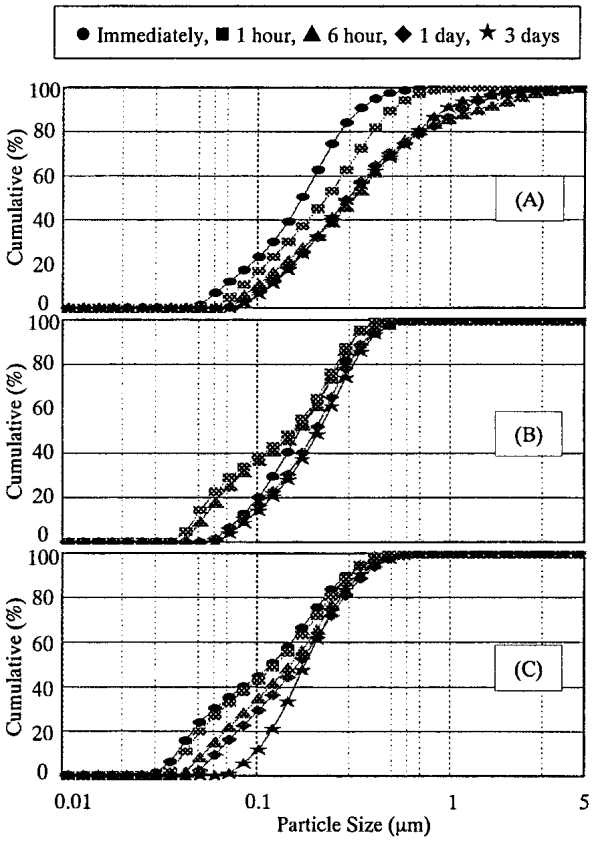

Fig. 7. Changes in particle size distribution patterns of β-CD·10.5H_2O GM filtrates at 30°C in water (A), in 0.1% HPMC solution (B), and in 0.1% PVP solution (C).

suspension because of the steric hindrance between the adsorbed polymer layers (27,28). It can be considered that polymers would adsorb on the surface of drug particle and prevent the aggregation of fine particles. Consequently, the stability of PRK fine particles in aqueous solution was improved by the addition of a water-soluble polymer.

CONCLUSION

Cogrinding with β-CD of high water content was found to be an effective method of preparing drug fine particles at the submicron level. Cogrinding with β-CD of different water

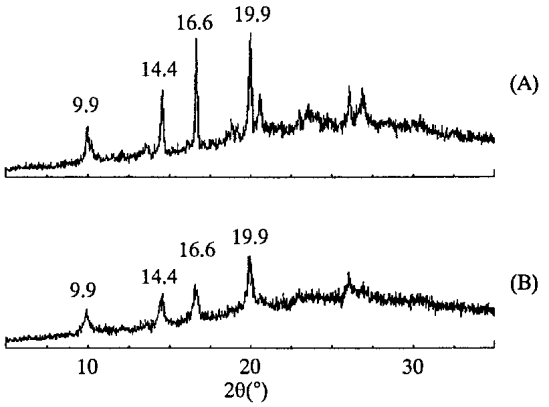

Fig. 6. PXRD patterns of PRK particles collected on the membrane filter (0.1 μm). (A) PRK crystals and (B) PRK fine particles in the suspension of β-CD·10.5H_2O GM (0.1–0.8 μm).

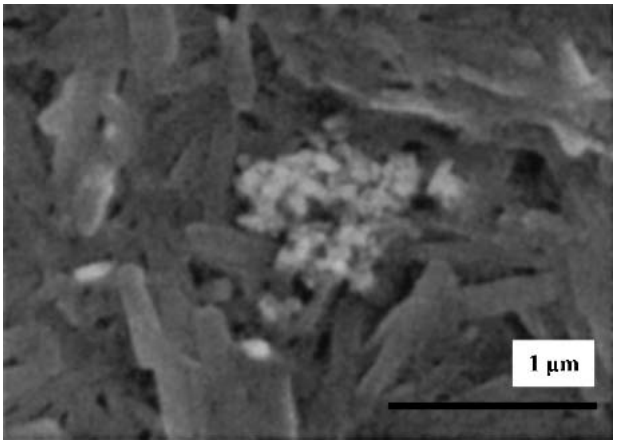

Fig. 8. SEM Photograph of PRK fine particles (0.2–0.8 μm) on membrane filters.

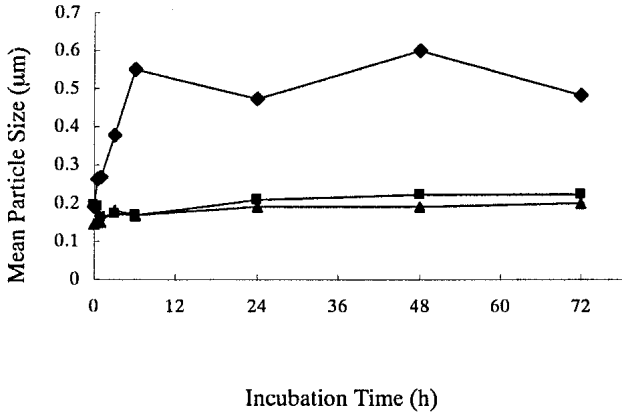

Fig. 9. Changes in mean particle size of PRK fine particles in β-CD·10.5H_2O GM filtrates at 30°C in water (◆), in 0.1% HPMC solution (■), and in 0.1% PVP solution (▲).

content resulted in GMs of remarkably different characteristics. Water molecules should act as a lubricant at the molecular level during the cogrinding process, resulting in improved grinding efficiency. Moreover, water would bind the drug and β-CD molecules and cause some changes at the surface, such as deformation or partially inclusion phenomenon, which might lead to the formation of fine drug particles. We speculated that the atmosphere in the grinding process markedly affected the surface characteristics of particles formed, although the influence of the water involved in the cogrinding process remains to be further investigated.

Furthermore, as for a possible mechanism of the fine-particle formation, we speculated that ultrafine PRK crystals would be dispersed in amorphous β-CD matrix. After dispersing the GM in water, β-CD dissolved, and submicron size of PRK crystals were released in the medium.

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