

Nanoparticle Formation of Poorly Water-Soluble Drugs from Ternary Ground Mixtures with PVP and SDS

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Received October 2, 2002; accepted November 16, 2002

Poorly water-soluble drugs N-5159, griseofulvin (GFV), glibenclamide (GBM) and nifedipine (NFP) were ground in a dry process with polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate (SDS). Different crystallinity behavior of each drug during grinding was shown in the ternary Drug/PVP/SDS system. However, when each ternary Drug/PVP/SDS ground mixture was added to distilled water, crystalline nanoparticles which were 200 nm or less in size were formed and had excellent stability. Zeta potential measurement suggested that the nanoparticles had a structure where SDS was adsorbed onto the particles that were formed by the adsorption of PVP on the surface of drug crystals. Stable existence of crystalline nanoparticles was attributable to the inhibition of aggregation caused by the adsorption of PVP and SDS on the surface of drug crystals. Furthermore, the electrostatic repulsion due to the negative charge of SDS on a shell of nanoparticles could be assumed to contribute to the stable dispersion.

Key words poorly water-soluble drug; nanoparticle; ground mixture; polyvinylpyrrolidone (PVP); sodium dodecyl sulfate (SDS)

For the improvement of the aqueous solubility and oral absorption of poorly water-soluble drugs, co-grinding method with water-soluble polymers and surfactants is one of the useful pharmaceutical techniques.^{1–7} According to Yamada *et al.*,⁸ the solubility of KCA-098, a poorly water-soluble drug, was improved by co-grinding with hydroxypropyl cellulose and polyvinylpyrrolidone. Solubility enhancement was due both to the amorphization of the drug and to the formation of submicron particles. The co-grinding technique has many advantages, that is, it can be carried out easily and economically, it can be conducted without organic solvents, and stable amorphous state can be obtained.^{9–13} According to Sugimoto *et al.*,¹⁴ nifedipine partly maintained crystallinity in the co-ground mixture prepared with nifedipine, polyethylene glycols and hydroxypropyl methylcellulose in the presence of water. The co-ground mixture showed remarkable improvement of solubility and showed same bioavailability as nifedipine solution of PEG 400 by oral administration to beagle dogs. When the co-ground mixture was added into water, coacervate-like fine particles (50–200 nm) which included crystalline nifedipine were formed. Liversidge *et al.*¹⁵ reported that crystalline nanoparticles formed by wet co-grinding of danazol with polyvinylpyrrolidone showed remarkable improvement in absorption. These results indicate that oral absorption of poorly water-soluble drug can be improved by the formation of crystalline nanoparticles of drugs, and that there is no need to transform a crystalline drug into an amorphous drug which is physically unstable. In general the wet co-grinding method is more effective to reduce the particle size than the dry co-grinding method,⁹ however, a wet process cannot be applied to drugs that may be hydrolyzed by water. Thus in this study, we adopted more convenient dry co-grinding method to investigate the formation of drug nanoparticles having a good dispersibility and stability in distilled water, aiming to improve the aqueous solubility of four poorly water-soluble drugs. Furthermore, we investigated the structure and formation mechanism of the obtained nanoparticles.

Experimental

Materials N-5159, *N*-methyl-*N*-(4,6-dimethylpyrid-2-yl)-1-[2-(4-(3,4-dimethoxybenzoyl)piperazin-1-yl)ethyl]benzimidazole-2-carboxamide was synthesized by Nisshin Seifun Group Inc. (Japan). Griseofulvin (GFV), glibenclamide (GBM) and sodium dodecyl sulfate (SDS) were of reagent grade and purchased from Wako Pure Chemical Industries, Ltd. (Japan). Nifedipine (NFP) was obtained from Moebs S.A. (Spain). Polyvinylpyrrolidone (PVP K-17, ISP Japan Ltd., Japan) was used as received. All other chemicals used were of analytical reagent grade.

Preparation of Ground Mixtures In ternary system, 2.5 g physical mixture (PM) of a drug (0.5 g), PVP K-17 (1.5 g) and SDS (0.5 g) was ground at room temperature by means of a vibrational rod mill (TI-500ET, CMT CO., Ltd., Japan). In binary systems, 2.5 g physical mixture of drug (0.625 g) and PVP K-17 (1.875 g) or drug (1.25 g) and SDS (1.25 g) was ground by the same method as described above. The grinding times that the mean particle size of nanoparticles obtained from the ternary ground mixtures showed minimum value were 150 min for N-5159 and GFV, and 30 min for GBM and NFP. Various ground mixtures were prepared at these grinding time. (Fig. 1).

Powder X-Ray Diffraction Measurement Powder X-ray diffraction was performed with Rigaku Miniflex diffractometer (Rigaku Corporation, Japan). Measurements were performed at 30 kV voltage, 15 mA current, a scanning angle from 2 to 35°, a scanning speed of 4° min⁻¹ and a radiation source of CuK α .

Measurement of Particle Size Distribution The volumetric particle size distribution for each suspension was determined by dynamic light scattering on a Microtrac[®] UPA (UPA 150, Nikkiso Co., Ltd., Japan). The mean particle size represented volume mean diameter determined from the volumetric particle size distribution.

Solubility Determination Each specimen containing 10 mg of drug was dispersed into 20 ml of distilled water. The dispersions in test tubes were shaken for 4 h at 150 strokes/min in a water bath thermostatted at 37°C. The dispersions that stable nanoparticles formed 4 h later were filtered through a membrane filter (0.2 μ m GD/X filter, Whatman Inc., U.S.A.) and the filtered solutions were appropriately diluted with the HPLC mobile phase solution. The drug concentrations in the solution were determined by HPLC (LC-6A, Shimadzu Co., Japan). The mobile phase was delivered at a flow rate of 1.0 ml/min through a L-Column[®] ODS (4.6 mm i.d. \times 15 cm; CERI, Japan) at 40°C. To determine the concentration of N-5159, GFV, GBM and NFP, acetonitrile/distilled water/phosphoric acid (50/150/1, 80/120/1, 110/90/1, 80/120/1, v/v/v) were used as a mobile phase, respectively. The detection wavelength was 254 nm.

Zeta Potential Measurement A zeta potential for each suspension was determined using a ZetaPALS[®] (Nikkiso Co., Ltd., Japan).

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Results and Discussion

Nanoparticles Formation by Ternary Drug/PVP/SDS System

In order to improve the solubility of four poorly water-soluble drugs, N-5159, GFV, GBM and NFP (Table 1), each drug was ground in a dry process with PVP and SDS by means of a vibrational rod mill. For each drug, a binary ground mixture, a ternary physical mixture and a ternary ground mixture were prepared by the procedures shown in Fig. 1. In order to determine the suitable mixing ratio of the ternary ground mixture, we evaluated drug solubility of various ground mixtures prepared by varying the PVP ($X=1, 3, 5, 8$) and the SDS ($Y=1, 2, 3$) in the Drug/PVP/SDS ($1 : X : Y$) system. We chose the ratio of $1 : 3 : 1$ (Drug/PVP/SDS, by weight), which showed the highest solubility of the drug while minimizing the quantity of additives.

In each case, the intact drug, the drug ground by itself, the binary ground mixtures, the ternary physical mixture, and the ternary ground mixture were added to distilled water to become a drug concentration of $500 \mu\text{g/ml}$, and the obtained suspensions were shaken for 4 h at 37°C . After the resulting suspension had been passed through a $0.2 \mu\text{m}$ filter, the drug concentration in the filtrate was determined. Figure 2 shows the drug concentration in the filtrate of intact drug and 5 types of sample obtained from each case. In every case, the filtrate obtained from the ternary ground mixture was colloidal and the concentration of each drug in the filtrate was found to be markedly high with values of $440\text{--}500 \mu\text{g/ml}$. This result was considered to be due to the formation of particles of less than $0.2 \mu\text{m}$ in the ternary ground mixture suspensions. The particle size distribution patterns of the intact suspension without filtration, which was obtained by dispersing the ternary ground mixture of each drug into distilled water are shown in Fig. 3. The mean particle size of colloidal particles obtained from the ternary ground mixtures of N-5159, GFV, GBM and NFP were 119, 103, 149, and 162 nm, respectively. Thus the formation of nanoparticles in each drug was confirmed. In contrast, the mean particle size of particles formed in the binary Drug/PVP and the Drug/SDS ground mixtures was $1110\text{--}4000 \text{ nm}$ and $378\text{--}1240 \text{ nm}$ respectively, remarkably large sizes compared to the ternary ground mixtures. It was considered to be attributable to the recrystallization of amorphous N-5159 in distilled water that the solubility of N-5159 in the solution obtained from the N-5159/PVP binary ground mixture was low as shown in Fig. 2. These results demonstrate that co-grinding methodology by the ternary Drug/PVP/SDS system was specific to form nanoparticles of 200 nm or less.

In order to evaluate the stability of nanoparticles obtained from the ternary ground mixture of each drug, an aqueous suspension of the ground mixture was kept at 37°C for 4 h and the variation of mean size with time was examined. Figure 4 shows the variation of the mean size of nanoparticles of each drug as a function of storage time. Slight increase (7–20%) of particle size was observed during the first 1 h of the storage, while no drug showed significant variation in mean particle size after 1 h.

To evaluate the crystallinity of the drug in the nanoparticles obtained from the ternary Drug/PVP/SDS ground mixtures, the powder X-ray diffraction pattern of the nanoparticles was determined. At first, the aqueous suspension of the ground mixture was passed through a filter with $0.45 \mu\text{m}$

Table 1. Physicochemical Properties of Various Poorly Water-Soluble Drugs

	Solubility ($\mu\text{g/ml}$) ^{a)}	Mean particle size (μm)
N-5159	46.9	85.1
Griseofulvin (GFV)	13.3	7.6
Glibenclamide (GBM)	1.1	17.1
Nifedipine (NFP)	19.2	54.6

a) Solubility in distilled water at 37°C .

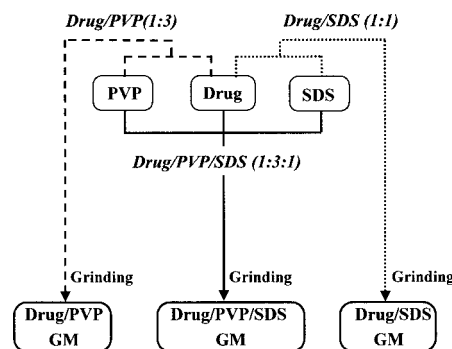


Fig. 1. Preparation Procedures of the Binary and Ternary Ground Mixtures

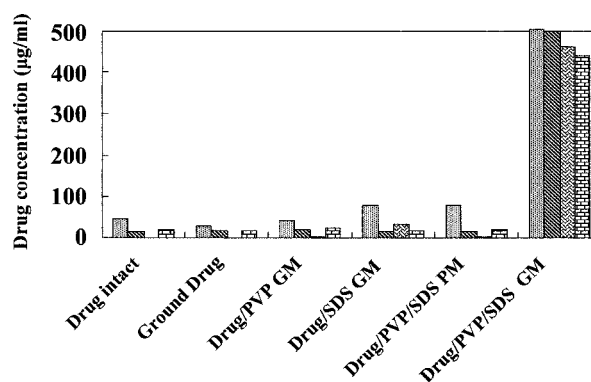


Fig. 2. Variation in the Amount of Drug Fine Particles ($\leq 0.2 \mu\text{m}$) after Dispersing Various Ground Mixtures and Physical Mixture into Distilled Water at 37°C

■, N-5159; ▨, griseofulvin; ▩, glibenclamide; ▪, nifedipine.

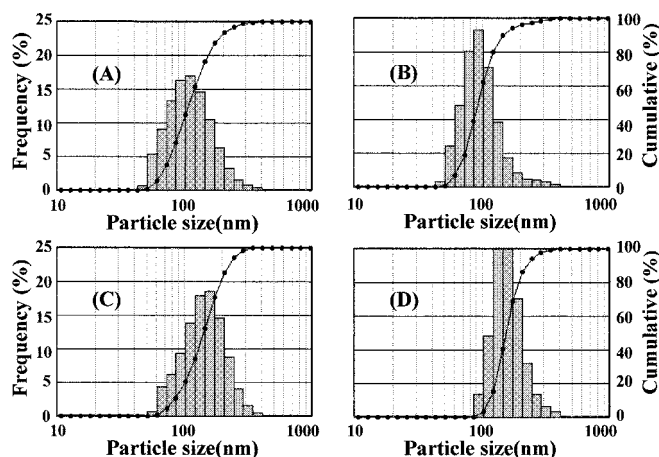


Fig. 3. Particle Size Distribution of the Colloidal Solutions Generated from Various Ternary Ground Mixtures

(A), N-5159/PVP/SDS ($1 : 3 : 1$) system; (B), GFV/PVP/SDS ($1 : 3 : 1$) system; (C), GBM/PVP/SDS ($1 : 3 : 1$) system; (D), NFP/PVP/SDS ($1 : 3 : 1$) system.

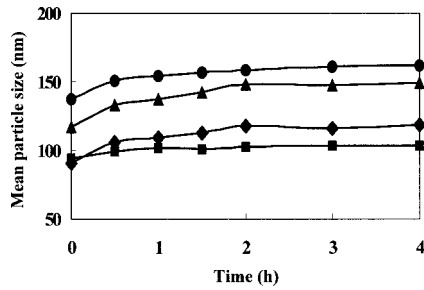


Fig. 4. Changes in the Mean Particle Size as a Function of Storage Time at 37 °C

—◆—, N-5159/PVP/SDS (1:3:1) system; —■—, GFV/PVP/SDS (1:3:1) system; —▲—, GBM/PVP/SDS (1:3:1) system; —●—, NFP/PVP/SDS (1:3:1) system.

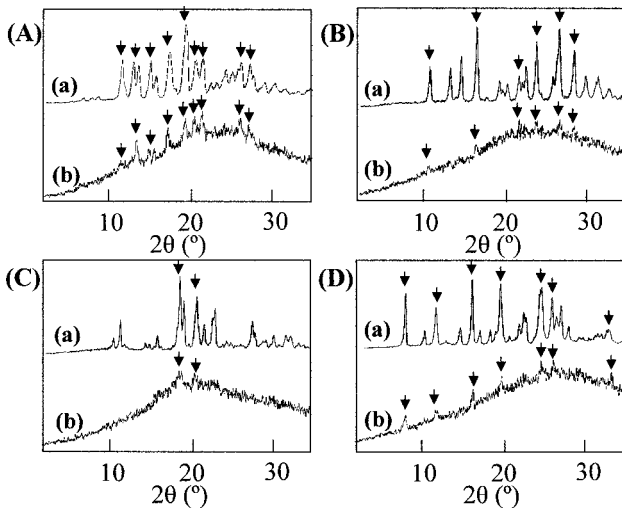


Fig. 5. Powder X-Ray Diffraction Patterns of Drug Nanoparticles Formed from Various Ternary Ground Mixtures

(a), drug intact; (b), drug nanoparticles formed from ternary ground mixture. (A), N-5159/PVP/SDS (1:3:1) system; (B), GFV/PVP/SDS (1:3:1) system; (C), GBM/PVP/SDS (1:3:1) system; (D), NFP/PVP/SDS (1:3:1) system.

pore-size, then through a filter with 0.1 μm pore-size to collect the nanoparticles on the filter. Figure 5 shows the powder X-ray diffraction patterns of the intact drug and the nanoparticles of each drug. The broad diffused diffractions observed in the nanoparticle were caused from the filter membrane, as the nanoparticles collected on the filter of 0.1 μm were directly used for the measurements. In each drug, the diffraction angles observed in the nanoparticles were in good agreement with those observed in drug crystals (indicated by arrows). These results indicated that the nanoparticles obtained from the ternary ground mixture included crystalline drug. Thus it was clarified that crystalline nanoparticles with good stability formed by adding the ternary Drug/PVP/SDS ground mixture into distilled water.

Structure of Nanoparticles Obtained from Ternary Ground Mixtures In order to examine the physical properties of the surface of the nanoparticles obtained from the ternary ground mixture, the zeta potential of the nanoparticles was determined for various ground mixtures, which were prepared by varying the SDS weight ratio (X) in the N-5159/PVP/SDS (1:3:X) system. Figure 6 shows the zeta potential values of nanoparticles as a function of SDS weight ratio. The zeta potential values of the ground mixture with

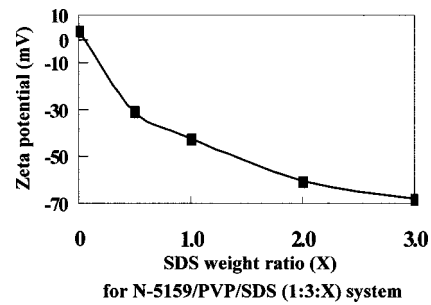


Fig. 6. Variation of Zeta Potential of Colloidal Solutions as a Function of Weight Ratio (X) of SDS for N-5159/PVP/SDS (1:3:X) System

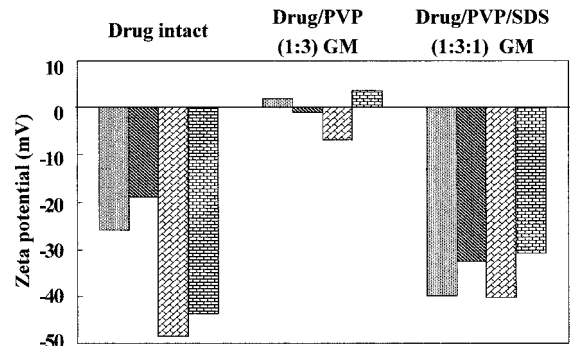


Fig. 7. Effect of Additives on Zeta Potential of Suspension Formed from Drug/PVP (1:3) and Drug/PVP/SDS (1:3:1) Ground Mixtures

▨, N-5159; ▩, griseofulvin; ▧, glibenclamide; ▦, nifedipine.

PVP (the sample of X=0) was determined as +2 mV, while the zeta potential value of intact N-5159 without any additives was -25 mV. This result was considered to indicate that the surface charge in N-5159/PVP system changed to neutral because PVP was adsorbed onto N-5159 crystal surface, which had been charged negatively.¹⁶⁾ In contrast, the zeta potential value of the ground mixtures prepared using with both PVP and SDS (X=0.5—3.0) changed to negative by the addition of SDS, that is, the zeta potential value decreased gradually with increasing weight ratio of SDS. These results were considered to result from the increased amount of SDS adsorbed onto the surface of nanoparticles.¹⁶⁾ Figure 7 shows the zeta potential values of the aqueous suspensions of intact drug, Drug/PVP ground mixtures and Drug/PVP/SDS ground mixtures for each drug.

The zeta potential of intact drug varied for each drug; values for N-5159, GFV, GBM and NFP were -25, -19, -48 and -44 mV, respectively. In contrast, the zeta potential values of the Drug/PVP ground mixtures were -7—+3 mV and almost electrically neutral. The zeta potential values of the Drug/PVP/SDS ground mixtures, however, were -40—-30 mV, showing homogeneous surface conditions compared to the intact drugs. Regardless of the surface potential of intact drug, the value of the surface potential changed to a certain range accompanying nanoparticle formation in the Drug/PVP/SDS system. The surface of the nanoparticles seemed to have a structure that SDS was adsorbed to the surface independently from drug type. From these results, we might presume that the nanoparticles obtained from the ternary Drug/PVP/SDS ground mixture have a structure where SDS was adsorbed onto the particles which were

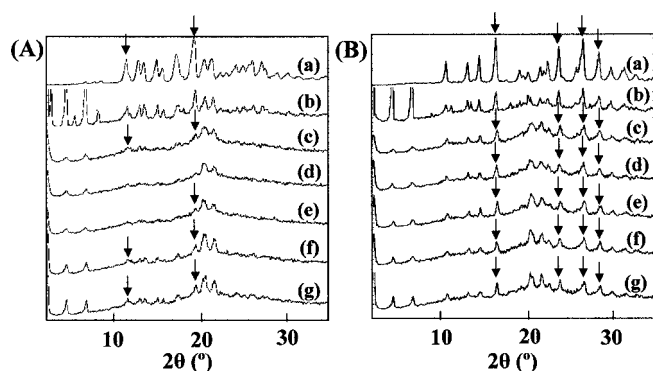


Fig. 8. Changes in the Powder X-Ray Diffraction Patterns during Grinding of N-5159/PVP/SDS (A) and GFV/PVP/SDS (B) Systems

(a), drug intact; (b), physical mixture; (c), co-ground for 10 min; (d), co-ground for 20 min; (e), co-ground for 30 min; (f), co-ground for 60 min; (g), co-ground for 150 min.

formed by adsorption of PVP on the surface of drug crystals.

Formation Mechanism of Nanoparticles Obtained from Ternary Ground Mixtures To investigate the mechanism of nanoparticles formation in the ternary Drug/PVP/SDS system, we examined the change of drug crystallinity in the ground mixtures and the variation of the mean particle size of nanoparticles formed by dispersing ground mixtures into distilled water. Figure 8 shows the changes of the powder X-ray diffraction pattern of the ternary ground mixtures of N-5159 and GFV with various grinding times. In the N-5159 ground mixture (A), the intensities of the X-ray diffraction peak of N-5159 crystals (indicated by arrows) gradually decreased with grinding and in 20 min ground mixture (d), no diffraction peak of N-5159 crystals was observed. These results demonstrated that with prolongation of grinding, crystalline N-5159 was gradually amorphized. In 30, 60 and 150 min ground mixtures (e, f, g), however, the intensities of diffraction peaks of N-5159 crystals gradually increased. As the temperature of the grinding cell was raised from 25 to 70 °C when the grinding time reached 150 min, we carried out the temperature controlled grinding at 30 °C. By the temperature controlled grinding, amorphous N-5159 was stably obtained even after long grinding time. Differential thermal analysis for the N-5159/PVP/SDS ground mixture represented the crystallization of amorphous N-5159 during heating process. From these results, it seems likely that appearance of X-ray diffraction peak of N-5159 crystals with long period of grinding (Fig. 8, (A) e–g) was attributable to the crystallization of amorphous N-5159 due to the heat produced by grinding.¹⁷⁾

In the GFV ground mixture (B), however, a remarkable change in the peak intensities of GFV crystal was not observed with prolongation of grinding, it was presumed that the micronized GFV crystallites were formed during grinding with PVP and SDS and the obtained microcrystals were dispersed in the additives.

Figure 9 shows the changes in the mean particle size of nanoparticles obtained from the N-5159 ground mixtures (A) and the GFV ground mixtures (B) which were prepared with various grinding times. The mean particle sizes of both ground mixtures decreased with prolongation of grinding time, and the mean particle sizes showed the smallest value when the sample was ground for the longest grinding time of

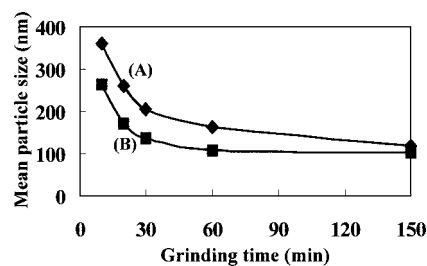


Fig. 9. Changes in the Mean Particle Size of Nanoparticles as a Function of Grinding Time for N-5159/PVP/SDS (A) and GFV/PVP/SDS (B) Systems

150 min. This was considered to result from the formation of drug microcrystals obtained by grinding, even though two drugs, N-5159 and GFV, represented different behavior in powder X-ray diffractogram.

The solubility, molecular arrangement and precipitation kinetics of each drug would affect the nanoparticle formation in the suspensions. Simonelli *et al.*¹⁸⁾ also reported that the adsorption of PVP to the particle surface in solution could inhibit the aggregation of the crystals. It may be concluded that when the ternary ground mixture containing micronized drug crystallites was dispersed in distilled water, nanoparticles were formed because PVP molecules dissolved in distilled water were quickly adsorbed on the surface of the drug microcrystals to inhibit hydrophobic aggregation among drug microcrystals. Also, we concluded that the electrostatic repulsion from the negative charge of the SDS that was adsorbed onto the surface of the nanoparticles contributed to the good dispersion stability of the obtained nanoparticles.

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