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Formation mechanism of colloidal nanoparticles obtained from probucol/PVP/SDS ternary ground mixture

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Abstract

The purpose of this study was to investigate the formation mechanism of colloidal nanoparticles after dispersion of probucol/polyvinylpyrrolidone (PVP)/sodium dodecyl sulphate (SDS) ternary ground mixture (GM) into water. Probucol, PVP and SDS were mixed at a weight ratio of 1:3:1 and ground for 30 min with a vibrational rod mill. The morphology and physicochemical properties were investigated through high resolution scanning electron microscopy (SEM), environmental SEM, dynamic light scattering, ^{13}C NMR and zeta potential measurements. SEM images confirmed the presence of 20 nm size primary particles in the GM powder of probucol/PVP K17/SDS. Spherical nanoparticles with a size of around 100 nm, formed after dispersion of the GM into water, suggested an agglomeration of the primary particles. A further agglomeration of around 160 nm was observed with the stability experiment. Zeta potential and particle size measurements using latex beads revealed that PVPK 17/SDS complex was adsorbed on the probucol particle surface forming a layered structure. A similar agglomeration behavior was observed using the GM of probucol/PVP K12/SDS, though the molecular state of the PVPK 12/SDS complex at the particle surface was different from that of the PVPK 17/SDS complex. ^{13}C NMR results suggested that intermolecular interactions between PVP K12 and SDS did not reach the same level as the interactions between PVP K17 and SDS. This study proposed a formation mechanism of colloidal nanoparticles.

Keywords: Nanoparticle; Grinding; Probucol; Polyvinylpyrrolidone; Sodium dodecyl sulphate; Environmental scanning electron microscopy

1. Introduction

Conventional grinding of active pharmaceutical ingredients commonly results in particles much greater than 1 μm due to their agglomeration. Wet co-grinding technique has been developed to enable the preparation of grinding-induced drug nanoparticles (Liversidge and Cundy, 1995; Wua et al., 2004; Lee et al., 2005). Nanocrystal formulation has been reported to show remarkably improved oral absorption of poorly water-soluble drugs (Wiedman et al., 1997; Merisko-Liversidge et al., 1996). However, the wet process could not apply on drugs that are easily degraded by hydrolysis. The storage condition of the sample at liquid state is also not favorable. Compared with the wet grinding process, dry co-grinding process showed some advantages for solid pharmaceuticals due to its simple preparation process without using organic solvent.

For the effective size reduction of the drug particles, water-soluble polymers and surfactants have been used as additives to inhibit the particles' agglomeration and improve the physicochemical properties of the drug (Leuner and Dressman, 2000). Polyvinylpyrrolidone (PVP), a water-soluble polymer, and sodium dodecyl sulphate (SDS), an anionic surfactant, have been used in a variety of pharmaceutical formulations (Behn, 2000; Kibbe, 2000). Drug–PVP and drug–SDS binary grinding have been used to improve stability and dissolution properties of several kinds of hydrophobic drugs, though the obtained particles easily agglomerated after storage of the suspension (Sugimoto et al., 1998; Gohel and Patel, 2000). The combined use of a polymer and a surfactant contributed to the effective stabilization of solid particles by the adsorption of a polymer–surfactant complex on the particle surface (Shimabayashi et al., 1997; Esumi et al., 2000; Lauten et al., 2000). The ternary system of a poorly water-

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soluble drug with polymer and surfactant should be a potential candidate for drug nanoparticle formation and stabilization.

Ground mixture (GM) of the poorly water-insoluble drug/PVP K17/SDS has been reported to form drug nanoparticles with good stability when the GM was dispersed into distilled water (Itoh et al., 2003; Pongpeerapat et al., 2004). Solid-state ^{13}C NMR studies revealed that grinding-induced solid-state interactions of probucol–PVP and PVP–SDS contributed to the effective size reduction and production of the drug nanocrystals in the ternary GM (Pongpeerapat et al., 2006). To evaluate the mechanism of drug nanoparticle formation, further investigation about the formation mechanism of a stable colloidal suspension after dispersion of the GM into water was necessary.

The purpose of this study is to investigate the formation mechanism of colloidal nanoparticles obtained after dispersion of the probucol/PVP/SDS ternary GM into water. Probucol, which has been used as an anticholesterol drug, was used as a model poorly water-soluble drug. The size reduction seems to improve the dissolution and the subsequent absorption from intestine. High resolution scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) was used to investigate the morphology of the nanoparticles. High resolution SEM has the advantage of distinguishing the morphology of extremely small particles in the GM powder. ESEM provides not only the morphological, but also the supportive information of the existence of nanoparticles in suspension state (Erickson et al., 2005; Laskin et al., 2006). The role of PVP and SDS on the formation of drug nanoparticles was discussed in terms of changes in particle size and magnitude of zeta potential. The correlation between particle size and charges of nanoparticles in the suspension provides detailed information on the nanoparticle formation. A mechanism regulating the agglomeration and stabilization of colloidal nanoparticles obtained by the ternary-grinding method is proposed.

2. Materials and methods

2.1. Materials

Probucol was supplied by Daiichi Pharmaceutical Co. Ltd. (Japan). Polyvinylpyrrolidone, PVP K12 (Kollidon® 12 PF, $M_w \sim 2500$) was obtained from BASF Japan Ltd. and PVP K17 (Plasdone® C15, $M_w \sim 10{,}000$) was acquired from ISP Technologies, Inc. (USA). Sodium dodecyl sulphate was purchased from Wako Pure Chemical Industries Ltd. (Japan). Polystyrene latex standard particles (100 ± 3 nm) were obtained from STADEX, JSR Co., Japan. All other chemicals were of reagent grade and used as received.

2.2. Preparation of ground mixtures (GMs)

Probucol (0.500 g), PVP (1.500 g) and SDS (0.500 g) (weight ratio of 1:3:1) were physically mixed in a glass vial using a vortex mixer (physical mixture, PM). For the preparation of ternary ground mixtures, the PM was ground in a vibrational rod mill (TI-200, Heiko Seisakusho, Japan) for definite time intervals. The grinding cell and rod were made of aluminium oxide. For the binary system, probucol (0.625 g) and PVP (1.875 g) or probucol (1.250 g) and SDS (1.250 g) were ground by the same method as described above. Since pH values of the ternary PMs and GMs were close to that of water (pH 5.54), there is no effect of PVP on pH values of the sample solutions.

2.3. Preparation of solvent-evaporated samples

Probucol (2.000 g), PVP (6.000 g) and SDS (2.000 g) (weight ratio of 1:3:1) were dissolved in ethanol (200 mL). The ethanol was evaporated using a rotary evaporator under vacuum at 60 °C for 2 h. The solvent-evaporated sample was crushed, then dried in a vacuum chamber over P_2O_5 at 70 °C for 4 h. The dried solvent-evaporated sample was pulverized and passed through a 0.2-mm sieve.

2.4. High resolution scanning electron microscopy (SEM) analysis

Scanning electron microscopy was performed using a Nova 200 NanoLab (FEI Company, Japan) operated at 3 kV. A sample was fixed to the SEM stage using a carbon paste, then coated with a platinum sputter.

2.5. Environmental SEM (ESEM) analysis

The nanoparticles in the suspension of probucol/PVP K17/SDS 1:3:1 GM were observed using a Quanta FEG (FEI Company) environmental scanning electron microscope. The chamber was held constant at 3 °C. The images were taken at 4.5 Torr with an accelerating voltage of 20 kV and 3.0 Torr with an accelerating voltage of 15 kV.

2.6. Particle size analysis

The GM was dispersed into distilled water and then sonicated for 2 min to make the suspension. The drug amount in the suspension was fixed as 0.50 mg/mL. Particle size was determined by the dynamic light-scattering method using Microtrac UPA® (Nikkiso, Japan; measurement range: 0.003–6 μm) and by the light-scattering method using Microtrac FRA® (Nikkiso, Japan; measurement range: 0.1–700 μm).

2.7. Stability study

Stability studies of the GM suspensions were conducted by the particle size analysis after storage at 25 °C for 0 and 4 h, then 1, 3, 7, 14, 28 and 84 days.

2.8. Determination of zeta potential

A zeta potential for each suspension in distilled water was determined using NICOMP 380ZLS® (USA). The measurements were repeated three times and average values were calculated.

Table 1
Mean particle size of probucol particles obtained from the binary GM[a] in distilled water, 1.5 mg/mL PVP K17 solution and 0.5 mg/mL SDS solution, measured by FRA (0.1–700 μm)

	Mean particle size (μm)[b]
Probucol intact in distilled water	19.0 ± 2.6
Probucol/PVP K17 1:3 GM in distilled water	23.0 ± 0.3
Probucol/PVP K17 1:3 GM in SDS solution	29.4 ± 0.7
Probucol/SDS 1:1 GM in distilled water	23.8 ± 0.4
Probucol/SDS 1:1 GM in PVP K17 solution	27.3 ± 0.5
Probucol/PVP K17/SDS 1:3:1 GM in distilled water	0.090 ± 0.002[c]

[a] Grinding time 30 min.
[b] Results are expressed as mean ± S.D. ($n = 3$).
[c] Suspension obtained from the ternary GM and measured by UPA (0.003–6 μm).

2.9. ^{13}C NMR spectroscopy

^{13}C NMR spectra were measured in triplicate on a JEOL JNM-LA500 spectrometer (JEOL, Japan) operating at 500 MHz. The measurement conditions were as follows: a temperature of 25 °C, a pulse width of 90°, a relaxation delay of 7.0 μs, and a scan of 2.0333 s for 4906 times. Tetramethylsilane was used as internal standard.

3. Results and discussions

3.1. Role of PVP and SDS on the dispersed state of probucol binary GMs

Combined use of both PVP K17 and SDS for co-grinding has been reported to play an important role for the drug nanoparticle formation, while the drug-additive binary system showed a limiting effectiveness for micronization when distilled water was used as the dispersing medium (Pongpeerapat et al., 2004). Binary GMs of probucol/PVP K17 or probucol/SDS were dispersed into SDS or PVP solution to investigate the effect of PVP and SDS on the grinding-induced probucol nanoparticle formation (Table 1). All systems of binary GMs showed no nanoparticle formation after dispersion into PVP or SDS solutions, indicating that both PVP and SDS were the required components during the co-grinding process for nanoparticle formation. Considering the results of our previous study, the mechanochemical stress during grinding particularly induced simultaneous interactions of probucol–PVP and PVP–SDS to generate nanometer-sized particles of probucol (Pongpeerapat et al., 2006). Rigid molecular interactions in the suspension were expected to contribute to the stabilization of nanoparticles through adsorption of PVP and SDS onto the drug nanoparticle surface in the suspension.

3.2. Nanoparticle morphology

The morphology of probucol nanoparticles obtained from the GM of probucol/PVP K17/SDS was observed using high resolution SEM (Fig. 1a). The high resolution SEM image confirmed

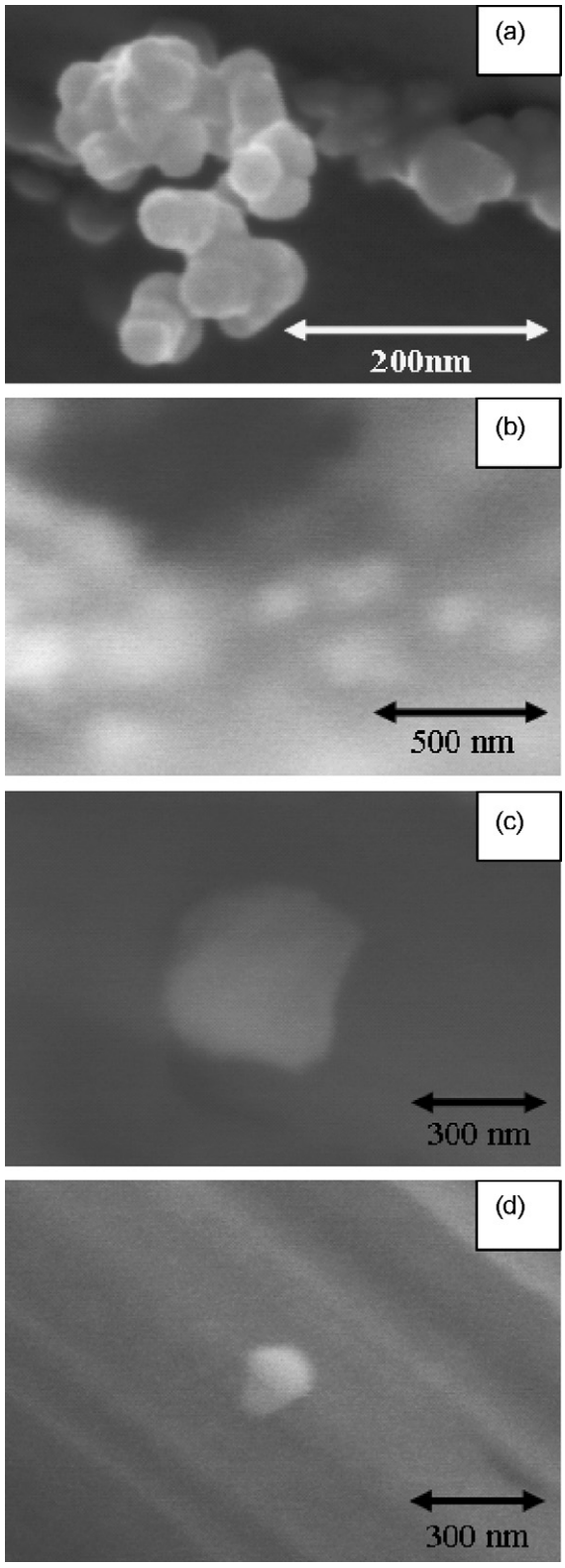


Fig. 1. SEM micrographs of probucol nanoparticles obtained from probucol/PVP/SDS 1:3:1 GM: (a) high resolution SEM of probucol nanoparticles in the probucol/PVP K17/SDS GM powder; (b) ESEM of probucol nanoparticles in the suspension prepared by dispersing the probucol/PVP K17/SDS GM into distilled water, imaged at 20 kV and 4.5 Torr; (c) ESEM of probucol nanoparticles at wet state, obtained from probucol/PVP K17/SDS, imaged at 15 kV and 3.0 Torr; (d) ESEM of probucol nanoparticles at wet state, obtained from probucol/PVP K12/SDS, imaged at 15 kV and 3.0 Torr.

the presence of primary nanoparticles in the GM powder. The agglomerates were composed of spherical nanoparticles with a size of around 20 nm. High resolution SEM micrograph of the GM powder of probucol/PVP K12/SDS also demonstrated that the agglomerates were composed of spherical nanoparticles with extremely small sizes of around 20 nm (Pongpeerapat et al., 2006).

The morphology of the suspended particles in water was investigated by ESEM. ESEM has been developed recently and successfully applied on the study of wet specimens. Unlike conventional high vacuum SEM, ESEM allowed for the retaining of water vapor pressure inside the sample chamber during the imaging process. Water vapor pressure and temperature inside the sample chamber could be varied and correlated with the relative humidity of real atmospheric conditions (Erickson et al., 2005; Laskin et al., 2006). In agreement with the previous results of the light-scattering measurements (Pongpeerapat et al., 2004), ESEM micrographs of the GM of probucol/PVP K17/SDS revealed the existence of spherical nanoparticles with a size of around 100 nm (Fig. 1b). Due to the lack of a conductive coating during the operation of ESEM and the movement of the nano-sized particles in the liquid state, the contrast of the images was low. However, the wet mode imaging by ESEM was helpful and showed distinct advantages in the investigation of the morphology of nanoparticles in liquid state. Higher contrast images were obtained when the vapor pressure was reduced from 4.5 to 3.0 Torr. Fig. 1c shows the morphology of an agglomerated particle imaged at 3.0 Torr with a size of around 300 nm. The liquid sample constantly lost water under conditions of reduced pressure, causing agglomerate formation through a combined nanoparticle interface. Fig. 1d shows the ESEM micrograph of the suspension of the GM powder of probucol/PVP K12/SDS after reduction of the vapor pressure to 3.0 Torr. The nanoparticles formed agglomerates of around 100 nm in size.

3.3. Physicochemical properties of drug nanosuspension prepared from the ternary GM

3.3.1. Changes in particle size after dispersion of the GM into water

Changes in particle size of the suspension obtained from GMs of probucol/PVP K12/SDS and probucol/PVP K17/SDS during storage are shown in Fig. 2. After the GM was dispersed into distilled water, drug nanoparticles with a mean particle size of 16 and 90 nm were formed in the suspensions prepared with PVP K12 and PVP K17, respectively. The small particles agglomerated with time. After storage for 4 days, of the suspension prepared with PVP K12, the particle size increased due to agglomeration of primary nanocrystals to 180 nm. In the case of the probucol suspension prepared with PVP K17, the increase of the particle size due to agglomeration was not so significant compared with the PVP K12 suspension. The results could raise some questions on how the nanoparticles were produced and stabilized in the suspension. Generally, fine particles in the suspensions were thermodynamically unstable due to their high energetic state. The small particles tended to reduce their surface free energy in order to reach a more thermodynamically

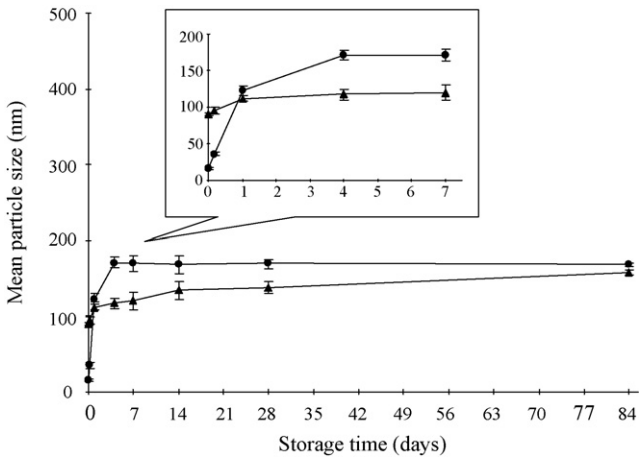


Fig. 2. Changes in mean particle size of the suspensions obtained from the ternary ground mixtures with storage time. Suspensions obtained from (●) probucol/PVP K12/SDS 1:3:1 GM, and (▲) probucol/PVP K17/SDS 1:3:1 GM. Inset shows the magnification of changes of the mean particle size for 7 days.

stable state by the formation of agglomerates. Interestingly, the particle size of the suspension obtained from the GM of probucol/PVP/SDS increased to some extent, and became stable at the size of less than 180 nm. This peculiar agglomeration behavior was observed only when the ternary GMs were dispersed into water.

A solvent-evaporated sample of the probucol/PVP K12/SDS system was prepared for comparison with the previous case. When the solvent-evaporated sample was dispersed into water, the solution was almost transparent at first. However, the solution became turbid very rapidly without stabilization. The mean particle size of the suspension just after dispersion of the evaporated sample into water was about 500 nm, then reached the detection limit of UPA® quickly within a few minutes. Crystal growth of the probucol crystals would occur during the storage period.

3.3.2. Surface state of the dispersed particles

Changes in the zeta potential of the suspensions obtained from the GM prepared with PVP K12 and PVP K17 are shown in Table 2. Zeta potentials of unprocessed probucol and binary GM of probucol/PVP K12 were −41.0 and −33.3 mV, respectively. The negative charge of zeta potential for unprocessed probucol was somewhat shielded in the presence of PVP K12. A change in zeta potential from −25.3 to −32.8 mV after storage for 1 day was observed in the suspension of the ternary GM prepared with PVP K12. This was accompanied by the increase in particle size from 16 to 123 nm (Fig. 2). On the contrary, the zeta potential was almost the same during the storage period, when the ternary GM was prepared with PVP K17. As the particle size of the ternary GMs stored for 1 day was almost the same, different adsorption states of PVP and SDS on the probucol surface seem to affect the zeta potentials depending on the difference of PVP molecular weight. The negative value observed in the ternary GM with PVP K17 was lower than that with PVP K12 at all storage conditions. Zeta potential of binary and ternary GMs prepared with PVP K17 were −10.3 and −17.7 mV, respectively, indicating that PVP

Table 2
Zeta potential of dispersed drug particles obtained from the GMs of probucol/PVP and probucol/PVP/SDS measured after dispersion into water

	Zeta potential (mV)[a]		
	After dispersion	1 day	7 days
Unprocessed probucol	−41.0 ± 1.6	–	–
Probucol/PVP K12 GM	−33.3 ± 1.6	–	–
Probucol/PVP K12/SDS GM	−25.3 ± 1.3	−32.8 ± 2.8	−30.2 ± 2.1
Probucol/PVP K17 GM	−10.3 ± 0.3	–	–
Probucol/PVP K17/SDS GM	−17.7 ± 2.2	−18.9 ± 2.0	−17.6 ± 0.3

[a] Results are expressed as mean ± S.D. ($n = 3$).

Table 3
Zeta potential and mean particle size of dispersed polystyrene latex particles[a]

Latex in	Zeta potential (mV)	Mean particle size (nm)
Distilled water	−50.94 ± 1.98	106 ± 1
PVP K12/SDS solution[b]	−41.22 ± 1.26	105 ± 2
PVP K17/SDS solution[b]	−27.54 ± 2.99	111 ± 1
PVP K12/SDS GM[c] solution	–	104 ± 2
PVP K17/SDS GM[c] solution	–	110 ± 2

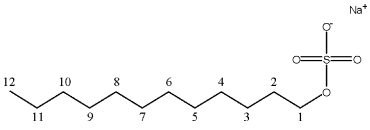
[a] Results are expressed as mean ± S.D. ($n = 3$).
[b] Concentration of PVP/SDS (3:1, w/w) was 2.0 mg/mL.
[c] PVP K17 and SDS were ground before the preparation of the solution.

K17 effectively shielded the negative charge of probucol, and that SDS affected the surface charges of probucol nanoparticles.

To investigate the effect of PVP grade on the molecular states of PVP and SDS on the particle surface, particle size and zeta potential measurements of polystyrene latex standard particles were carried out. The concentrated latex suspension was dispersed into distilled water, PVP K12/SDS (3:1) solution and PVP K17/SDS solution (2.0 mg/mL), respectively. As shown in Table 3, the zeta potential of PVP K12/SDS adsorbed latex particles showed larger negative values than that of latex particles which dispersed in PVP K17/SDS solution. Furthermore, the increase of the particle size indicated the formation of PVP K17/SDS complex layer on the latex surface, though that was not observed in the suspension of PVP K12/SDS solution. These results indicated that the interaction between PVP K12 and latex surface was too weak to form the layered complex structure on the latex surface. It seemed reasonable to consider that high molecular weight PVP could form complex with SDS and that the particle surface was covered by the complex accompanying the stabilization of nanosuspension. The difference in the adsorption states of PVP and SDS depending on the molecular weight of PVP influenced the zeta potential as shown in Table 3.

Differences in the surface state of probucol nanoparticles with PVP K17/SDS and with PVP K12/SDS were further investigated by ^{13}C NMR spectroscopy. ^{13}C NMR measurements have been used to study the polymer–surfactant interaction and to obtain information about the structure of the polymer–surfactant aggregate (Chari, 1991; Li et al., 1998). In this study, intermolecular interactions between SDS and either PVP K17 or PVP K12 were evaluated using ^{13}C NMR. Table 4 shows the chemical shifts of carbon atoms of SDS molecules in distilled water and PVP solutions. Two concentrations of SDS were used, 1.5 and 6.0 mg/mL, as the critical micelle concentration (CMC) of SDS at 20 °C was reported as 2.4 mg/mL (Behn, 2000). At a SDS concentration lower than the CMC (1.5 mg/mL), PVP remarkably influenced the upfield shift of SDS C1 carbon, which is directly attached to the sulphate group. Moreover, the presence of PVP influenced the downfield shift of the carbons at the alkyl chain, i.e. C2, C3, C11 and C12. Due to similar electronic environments

Table 4
^{13}C NMR chemical shift of the carbon atoms of SDS in distilled water and in PVP solutions



Carbon atoms of SDS	^{13}C NMR chemical shift of the carbon atoms of SDS (ppm ± S.D.[a] ($\Delta\delta$)[b])			
	Distilled water[c]	PVP K12 solution[c,d]	PVP K17 solution[c,d]	Distilled water[e]
C1	72.59 ± 0.00	72.25 ± 0.02 (−0.34)	71.88 ± 0.01 (−0.71)	72.29 ± 0.00 (−0.30)
C2	31.06 ± 0.00	31.36 ± 0.01 (0.30)	31.67 ± 0.01 (0.61)	31.70 ± 0.01 (0.64)
C3	27.67 ± 0.00	27.90 ± 0.01 (0.23)	28.14 ± 0.01 (0.47)	28.18 ± 0.02 (0.51)
C11	24.92 ± 0.00	25.08 ± 0.00 (0.16)	25.25 ± 0.01 (0.33)	25.32 ± 0.01 (0.40)
C12	16.28 ± 0.00	16.49 ± 0.00 (0.21)	16.62 ± 0.01 (0.34)	16.56 ± 0.01 (0.28)

[a] Results are expressed as mean ± S.D. ($n = 3$).
[b] $\Delta\delta$ represents the difference in chemical shifts of the corresponding solution from those of the SDS in distilled water.
[c] Concentration of SDS was 1.5 mg/mL.
[d] Concentration of PVP was 10.0 mg/mL.
[e] Concentration of SDS was 6.0 mg/mL.

and overlapping with the broad spectrum of PVP, the chemical shifts of the other carbon atoms of SDS molecules were difficult to resolve at low concentrations (Li et al., 1998; Roscigno et al., 2003). Significant changes in chemical shifts of SDS in the presence of PVP K12 or PVP K17 indicated the interaction between PVP and SDS. The upfield shift of C1 of SDS was attributed to the electrostatic interaction between the nitrogen in pyrrolidone ring of PVP and the negatively charged head group of SDS, while the downfield shifts of the other carbon atoms of SDS were due to the hydrophobic interaction between alkyl chains of SDS molecules. When the concentration of SDS was more than CMC, an upfield shift for C1 and downfield shifts for the remaining carbon atoms were also observed.

Changes of the chemical shifts of SDS carbons were much greater in the PVP K17 solution than in the PVP K12. The chemical shift of the SDS carbons in the PVP K17 solution, except for that of the C1, was close to that in the SDS micelle solution. These results suggested that the environmental state of all carbon atoms of SDS except C1 in PVP K17 solution was similar to that of the SDS micelle solution. C1 showed a remarkable change in chemical shift. This would be due to the intermolecular interaction between SDS head group and PVP K17. The structure of PVP K17/SDS complex was expected similar with the micelle-like aggregates of SDS. On the contrary, changes in the chemical shift of SDS carbons in PVP K12 solution were small compared to that observed in the PVP K17 solution. It was found that the chain length of PVP K12 was not long enough to form a micelle-like SDS–PVP aggregate structure. Results of NMR supported the different surface states of PVP and SDS depending on the molecular weight of PVP.

Spontaneous complexation of PVP and SDS, both through electrostatic and hydrophobic interactions in aqueous solutions has been recognized (Li et al., 1998; Nörenberg et al., 1999; Sukul et al., 2000; Misselyn-Bauduin et al., 2001; Roscigno et al., 2003). An electrostatic interaction should exist between the negatively charged head group of SDS and the nitrogen atom on the pyrrolidone ring of PVP (Li et al., 1998). In the polymer–surfactant system, the critical aggregation concentration (CAC), defined as the onset of the surfactant concentration when interaction between the surfactant and the polymer occurred, has been used to describe the interactions. The CAC of SDS was 0.08 mg/mL in the PVP solution, a value signifi-

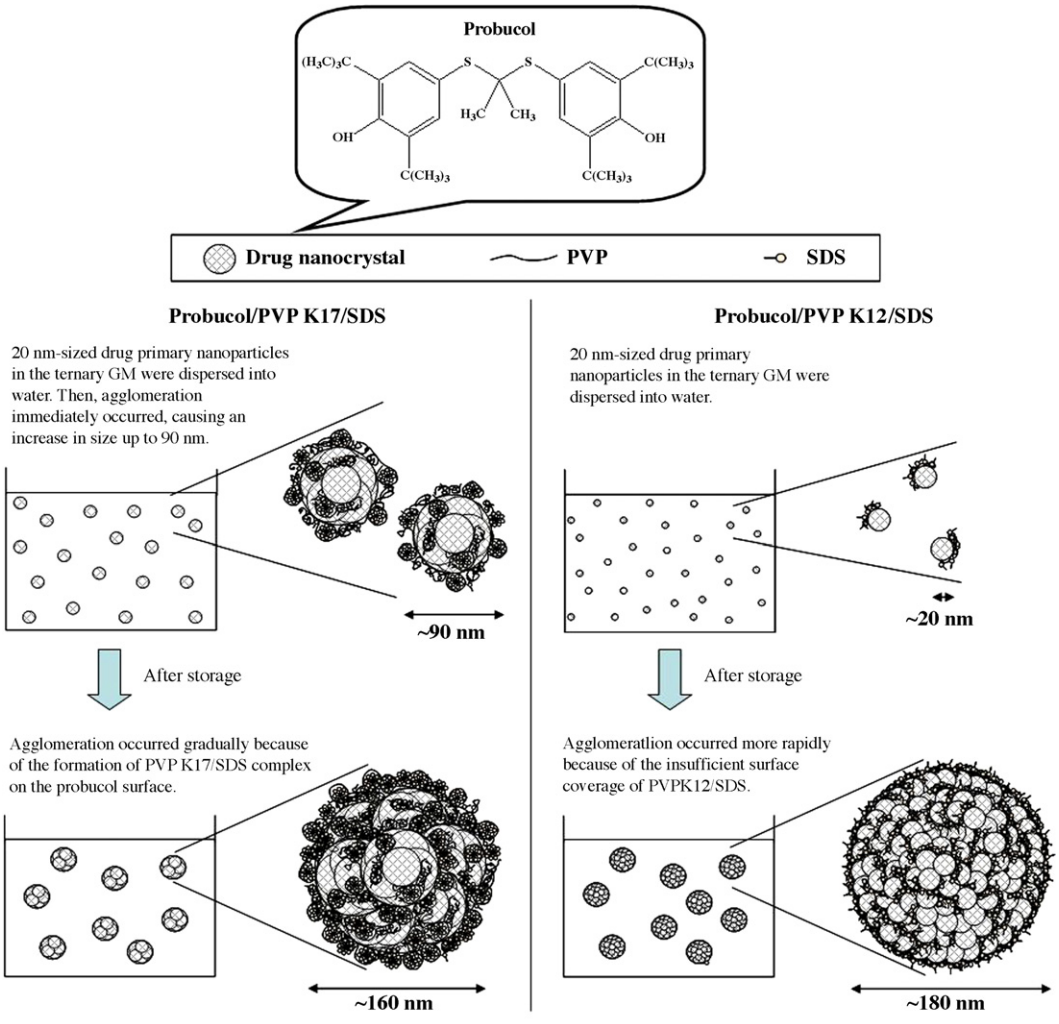


Fig. 3. Schematic representation of the formation mechanism of nanocrystalline drug particles from the probucol/PVP/SDS ternary GM.

cantly lower than the cmc (2.4 mg/mL at 20 °C). The CAC was independent from the concentration of the polymer (Nörenberg et al., 1999). Above the CAC, the PVP–SDS complex has been reported to form a "necklace structure" (Sear, 1998; Sukul et al., 2000) in the suspension. The concentration of SDS used was 0.5 mg/mL, when the ternary GM of probucol/PVP K17/SDS was dispersed into water, this suggests that PVP and SDS formed a necklace-structured complex in the suspension of the ternary GM.

3.3.3. Proposed mechanism of grinding-induced nanoparticle formation

A possible mechanism of drug nanoparticle formation is illustrated in Fig. 3. The results of SEM, zeta potential and liquid ^{13}C NMR measurements presented in this study were combined with the previous results of the solid-state ^{13}C NMR studies (Pongpeerapat et al., 2006). The solid-state ^{13}C NMR studies revealed that the grinding-induced solid-state interactions of probucol–PVP and PVP–SDS contributed to the effective size reduction and production of the drug nanocrystals. The SEM micrographs confirmed the presence of nanometer-sized drug nanocrystals (as small as 20 nm) as primary nanoparticles in the ternary GM. The formation mechanism of colloidal nanoparticles obtained from the probucol/PVP K17/SDS ternary GM was estimated as follows. By dispersing the GM of probucol/PVP K17/SDS into water, agglomeration of primary drug nanoparticles, which interacted with PVP K17/SDS complex as a necklace structure on the nanocrystalline surface, immediately occurred, causing a sudden increase in size up to 90 nm. The secondary nanoparticles reached a dynamic stabilization by further agglomeration, the particle size was less than 160 nm even after long time storage.

In the case of probucol/PVP K12/SDS, agglomeration of primary drug nanoparticles occurred more rapidly because of the insufficient surface coverage of PVP K12 and SDS on the probucol surface. The particle size of the secondary nanoparticles reached 180 nm after 4 days of storage, and then remained stable. Adsorption of PVP K12 on the surface of probucol nanoparticles should contribute to this stabilization as well.

4. Conclusion

The formation mechanism of colloidal nanoparticles prepared from the GM of probucol/PVP/SDS was evaluated by particle size analysis, zeta potential and ^{13}C NMR measurements. SEM micrographs confirmed the presence of nanometer-sized probucol nanocrystals as primary nanoparticles in the ternary GM. Agglomeration and subsequent stabilization behavior of the suspension from the ternary GMs with PVP K12 and PVP K17 were specifically observed using the ground samples. Molecular states of PVP and SDS on the probucol nanocrystals were well evaluated using latex particles. Differences in the interaction between PVP and SDS depending on the molecular weight of PVP would influence their absorption behavior on the probucol nanocrystals. Stabilization of probucol nanocrystals obtained from the GM of probucol/PVP K17/SDS was explained in terms of the formation of a PVP K17/SDS complex layer on the probucol surface as the layered structure. On the contrary, insufficient surface coverage of PVP K12 on the probucol nanocrystals would induce rapid agglomeration of primary nanoparticles in the case of probucol/PVP K12/SDS. Though PVP K12 and SDS did not form a layered structure on the particle surface, adsorption of PVP K12 on the surface of probucol nanoparticles contributed to the stabilization. It is speculated that the different surface coverage state as well as particle size should influence on probucol absorption when the further in vivo experiments are conducted.

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