ROLE OF POLYMER AND SURFACTANT ON PROBUCOL
NANOPARTICLE FORMATION FROM TERNARY CO-GRINDING

CHALERMPHON WANAWONGTHAI

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ABSTRACT

Recently, the use of nanoparticles as a drug delivery approach has been receiving a great attention. Dry grinding is considered to be a simple method for producing drug nanoparticles. However, the grinding of the drug substance alone is not effective enough to reduce the particle size because of the aggregation of the fine drug particles after grinding.

In order to obtain physically stable drug nanoparticles, co-grinding with additives such as polymers or surfactants is employed. The drug nanoparticle formation from ternary co-grinding of drug, water-soluble polymer and surfactant has been demonstrated. Solid-state NMR technique was utilized to evaluate the molecular interaction of ternary co-ground mixture consisting probucol, polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate (SDS). It was found that molecular interaction between probucol-PVP and PVP-SDS played an important role in nanoparticle formation by co-grinding. In this study, the further investigation was conducted in order to get advanced knowledge on drug nanoparticle formation from ternary co-grinding. Firstly, effect of polymer species on nanoparticle formation from ternary co-ground mixture of probucol/polymer/SDS was explored. Then, effect of charge of surfactant on probucol nanoparticle formation from probucol/PVP/surfactant was investigated. Finally, effect of surfactant on probucol nanoparticle formation from probucol/PVP/sodium alkyl sulfate co-ground mixture was also investigated.

Probucol was used as a model of poorly water-soluble drug. As a water-soluble polymer in the component, AQQAT® (hypromellose acetate succinate), Eudragit® L100 (methacrylic acid-methyl methacrylate copolymer), Kollicoat® IR (polyvinyl alcohol-polyethylene glycol graft copolymer), Plasdone® S630 (vinylpyrrolidone-vinyl acetate copolymer), polyethylene glycol 4000, polyvinyl alcohol and polyvinylpyrrolidone were used. As anionic surfactants, we used sodium alkyl sulfates with different chain lengths: sodium hexyl sulfate (C6S), sodium octyl sulfate (C8S), sodium dodecyl sulfate (C12S, SDS), sodium hexadecyl sulfate (C16S) and sodium octadecyl sulfate (C18S). Polyoxyethylene 20 stearyl ether (Brij® 78) and hexadecyltrimethylammonium bromide (CTAB) were used as non-ionic and cationic surfactants, respectively.
Probucol, polymer and surfactant were mixed at weight ratio of 1/3/1 in the study of polymer species and at weight ratio of 1/3/0.5 and 1/3/1 in the study of effect of surfactant charge. In the study of the surfactant chain length, the weight ratio of probucol/PVP/sodium dodecyl sulfate was varied between 1/3/0.1-1/3/1. The physical mixture (PM) was prepared by mixing the drug, polymer and surfactant for 5 min using a vortex mixer. To prepare ternary co-ground mixture (GM), PM was ground in a vibrational rod mill for 30-60 min. The physicochemical properties of GMs in solid state were investigated by powder X-ray diffraction (PXRD) and $^{13}$C solid-state NMR spectroscopy. Particle size measurement of co-ground mixture suspensions in water was performed by dynamic light scattering technique. To investigate the effect of charge of surfactants, the surface charge of probucol nanoparticles was determined by zeta potential measurement. Determination of percentage of probucol nanoparticle smaller than 0.8 µm was conducted by HPLC.

The effect of polymers species on probucol nanoparticle formation was evaluated. Probucol nanoparticles with the mean particle size smaller than 1 µm (around 90 - 600 nm) were obtained when all species of polymer were used. Since the particle size of unprocessed probucol was around 30 µm, the size reduction was dramatically induced by ternary co-grinding of probucol with polymer and SDS. Among the results, PVP K17 showed a superior particle size reduction over other polymers. The difference of mean particle size might be due to the unequal degree of strength in intermolecular interaction among the components in solid state.

Influence of surfactant charge on probucol nanoparticle formation from ternary co-grinding was investigated. Ground mixtures of probucol were obtained by co-grinding with PVP and anionic surfactant (SDS), cationic surfactant (CTAB) or non-ionic surfactant (Brij® 78). Probucol nanoparticles obtained using Brij® 78 had the average size around 150-180 nm, while those obtained using SDS and CTAB had smaller size of around 50-80 nm and 80 nm, respectively. Since the molecular interaction between PVP-Brij®, PVP-SDS and PVP-CTAB in water has been reported, grinding-induced intermolecular interaction among the components was expected to be the possible mechanism for probucol nanoparticle formation by grinding method. According to the results of zeta potential, the charge of the nanoparticle corresponded with surfactant used. It was speculated that surfactant existed at the surface of
probucol nanoparticles and involved in the prevention of agglomeration via electrostatic repulsion or steric hindrance.

The effect of chain length of sodium alkyl sulfate on probucol nanoparticle formation was further evaluated. Probucol nanoparticles of around 70-100 nm were obtained from probucol/PVP/sodium alkyl sulfate co-ground mixture at a weight ratio of 1/3/1. There was no significant difference in mean particle size of probucol nanoparticles obtained from using different species of surfactant at this weight ratio. However, the percentage of nanoparticle formation was dependent on surfactant species and weight ratio. The efficiency of nanoparticle formation, based on the quantitative determination of nanoparticles smaller than 0.8 µm, was in the order: C18S>C16S>C12S>C8S>C6S. For instance, when the C6S was used, percentage of nanoparticles was 1.3% at the weight ratio of 1/3/0.1. On the other hand, when C18S was used, percentage of nanoparticles was around 30% at weight ratio of 1/3/0.1. From this results, it was suggested that sodium alkyl sulfate with a longer alkyl chain length was more favorable to interact with PVP which resulted in the improved nanoparticle formation even if it was added in smaller amount.

$^{13}$C solid-state NMR measurement of probucol/PVP/sodium alkyl sulfate ternary mixtures was performed to investigate intermolecular interaction. Probucol and surfactant crystals were distinctly observed by NMR measurement while probucol crystal could not be detected by PXRD measurement. In each ternary co-grinding system, a certain amount of surfactant was needed to interact with PVP to induce probucol nanoparticle formation. In addition to the shifted peak due to this intermolecular interaction, the excess amount of surfactant crystal which did not involve in the interaction could be observed in NMR spectra of co-ground mixtures. At the weight ratio where excess amount of surfactant crystal appeared in NMR spectra, nanoparticle formation (ca. 100%) in water was observed. On the other hand, at weight ratio where excess amount of surfactant could not be observed in NMR spectra, percentage of nanoparticle formation was pretty low. These results suggested that surfactant was not only involving in the size reduction of probucol crystals during the grinding, but the excess amount of surfactant also required for the stabilizing nanoparticles after dispersing in water.
In conclusion, species of polymer and surfactant in ternary co-ground mixture exerted very important role on probucol nanoparticle formation. Percentage of probucol nanoparticle formation increased as the weight ratio of surfactant increased. Small amount of longer sodium alkyl sulfate yielded a higher percentage of nanoparticle formation. The results of solid-state NMR well explained the result of percentage of probucol nanoparticle formation. Excess amount of surfactant in the co-ground mixture was required to stabilize probucol nanoparticles in water.

These findings brought about the important aspects for the formulation optimization of drug/polymer/surfactant ternary co-grinding system. The appropriate selection of both species and quantity of additives was a necessary issue in producing the desired drug nanoparticles. This comprehensive knowledge on specific drug-additive interactions will contribute a more scientific basis for additives selection.
CHAPTER I
INTRODUCTION

Among the orally administered solid pharmaceutical dosage forms, the dissolution of drug molecule is the key determinant in the amount of drug to be absorbed. However, as reported by Takagi et al. 1), 30-40% of drugs on each list of the top 200 immediate-release drug products from the United States, Great Britain, Spain and Japan are categorized as practically insoluble drugs. The enhancement of drug solubility has been studied for many decades, and many techniques such as the complex formation with cyclodextrin 2,3), the solid dispersion of the drug with additives 4-7), water soluble prodrug formation 8,9) and water soluble salt formation 10,11) have been employed to increase the solubility of drugs. Additionally, according to the Noyes-Whitney equation, the particle size reduction i.e., the increase of the particle surface area is the effective method to increase the dissolution rate.

The grinding method is a simple way to reduce the size of particles. However, the grinding of the drug substance alone is not effective enough to reduce the particle size and to obtain a physically stable drug formulation 12). Addition of a water-soluble polymer and/or a surfactant as one of the components could resolve this problem and show satisfactory results. Several studies revealed that the co-grinding of a drug with additives induces the particle size reduction and enhances the dissolution rate 13-15). Mura et al. reported that co-grinding of glisentide with polyvinylpyrrolidone (PVP) evidently showed a better dissolution profile due to the size reduction, polymorphic transformation and amorphization 16). Mechanical stress during the grinding process induces distortion of particles, amorphization and molecular interaction between the drug and the additives 17-19).

From our previous study, it has been revealed that co-grinding of several poorly water-soluble drugs, such as griseofulvin, phenytoin and probucol, with PVP and sodium dodecyl sulfate (SDS) is an effective method to produce nanosuspensions of drug in aqueous medium. The average size of the drug nanoparticles measured in water was less than 200 nm, and stable at least for 2 weeks 20). Moribe et al. 21) also reported that using hydroxypropylmethylcellulose (HPMC) or methylcellulose (MC) yielded a smaller drug particles for drugs such as flurbiprofen those nanoparticles could not be
obtained from co-grinding with PVP. From these results, it should be emphasized that ternary co-grinding would be an effective method for drug nanoparticle formation.

Probucol is an anti-cholesterol drug. The solubility of probucol in water was estimated to be around 2-5 ng/mL at 25 ºC \(^{22}\). Thus, probucol showed a low absorption profile when orally administered. Zaitseva et al. \(^{23}\) showed that, the maximum blood plasma concentration after orally administration of 1 g probucol tablet to male patients was only 0.0003%. Therefore, probucol is a low solubility and low absorption drug according to the biopharmaceutical classification system \(^{24}\). The \textit{in vivo} absorption study of probucol nanoparticles prepared from co-grinding with different molecular weights of PVP and SDS was evaluated by Shudo et al. \(^{25}\). It was found that the co-ground mixture exhibited a superior absorption profile over unprocessed probucol. The absorption of probucol increased up to 5 to 40 times depended on the particle size of nanoparticles. Thus, nanoparticle formation of probucol by the co-grinding method might be a promising approach to improve its bioavailability when orally administered.

It has been reported that intermolecular interactions among the components, drug/polymer/surfactant, were hypothesized as the key factor of drug nanoparticle formation. Solid-state intermolecular interactions among probucol, several molecular weights of PVP and SDS had been investigated by Pongpeerapat et al. \(^{26}\). These authors reported that, from the \(^{13}\)C solid-state NMR results, the lower molecular weight PVP interacted with probucol and SDS stronger than the high molecular weight PVP when the ternary mixture was ground. As a result, the smaller probucol nanoparticles were obtained using low molecular weight of PVP.

Understanding the role of drug and additives on the solid-state formation of nanoparticles will provide crucial benefits in selecting appropriate additives and ratio of the components for producing drug nanoparticles. In this study, the further investigation was conducted in order to get advanced knowledge on drug nanoparticle formation from ternary co-grinding. Firstly, effect of polymer species on nanoparticle formation from ternary co-ground mixture of probucol/polymer/SDS was explored. Then, effect of surfactant charge on probucol nanoparticle formation from probucol/PVP/surfactant was investigated. Finally, the effect of alkyl chain length of surfactant on probucol nanoparticle formation from probucol/PVP/sodium alkyl sulfate co-ground mixture was also investigated.
CHAPTER II

MATERIALS AND EXPERIMENT

Materials

Structures and physicochemical properties of raw materials were shown in Table 1, 2 and 3.

Model drug probucol (Form I) was kindly supported from Daiichi-Sankyo Co., Ltd. (Japan). Probucol polymorph Form II was prepared by melting 3 g of probucol in hot air oven at 140°C for 30 min, and then rapidly cooled down by liquid nitrogen.

Seven water-soluble polymers were used in this study. AQt® (hypromellose acetate succinate, \( M_w \) 17,000 ~ 20,000) and Eudragit® L100 (methacrylic acid-methyl methacrylate copolymer (1:1), \( M_w \) 135,000) were obtained from Shin-Etsu Chemical Co., Ltd. (Japan) and Evonic Degussa Japan Co., Ltd. (Japan), respectively. Plasdone® C15 (PVP K17, \( M_w \) ~10,000) and Plasdone® S630 (vinylpyrrolidone-vinyl acetate copolymer (60:40), \( M_w \) 24,000 ~30,000) were obtained from ISP Japan Ltd. (Japan). Koliicoat® IR (polyvinyl alcohol-polyethylene glycol graft copolymer (75:25), \( M_w \) ~45,000) was obtained from BASF Japan Ltd. (Japan). Polyethylene glycol (\( M_w \) ~4,000) and polyvinyl alcohol (\( M_w \) 9,000 ~ 10,000) were purchased from Wako Pure Chemical Industries, Ltd. (Japan) and Sigma-Aldrich, Inc. (USA), respectively.

A cationic surfactant, hexadecyltrimethylammonium bromide (CTAB) and a non-ionic surfactant, polyoxyethylene 20 stearyl ether (Brij®) were purchased from Wako pure chemical Industries, Ltd. (Japan). As anionic surfactants, sodium hexyl sulfate (C6S) was purchased from ChemPur Feinchemikalien and Forschungsbedarf GmbH (Germany). Sodium octyl sulfate (C8S) and sodium octadecyl sulfate (C18S) were purchased from Sigma-Aldrich, Inc. (USA). Sodium dodecyl sulfate (C12S) and sodium hexadecyl sulfate (C16S) were purchased from Wako Pure Chemical Industries, Ltd. (Japan).

All chemicals were used as received.
Preparation of physical mixture (PM) and ground mixture (GM)

Probucol/polymer/SDS

In the study of effect of polymer species (section III-A) the weight ratio of probucol/polymer/surfactant was fixed at 1/3/1. The physical mixture (PM) was prepared by mixing for 5 min using vortex mixture. For the preparation of ternary ground mixture (GM), PM was ground in a vibrational rod mill (TI-500ET, CMT Co., Ltd.) for 30-60 min. When PEG was used, cryo-grinding with the assist of liquid N₂ was performed by a vibrational rod mill (TI-500ET, CMT Co., Ltd.).

Probucol/PVP/surfactant

In the study of effect of surfactant charge (section III-B) the weight ratio of probucol/polymer/surfactant was fixed at 1/3/0.5 and 1/3/1. Physical mixture and ground mixture were prepared at the same manner as stated in the above section. Co-grinding was conducted for 30 min.

Probucol/PVP/sodium alkyl sulfate

In the study of effect of chain length of sodium alkyl sulfate (section III-C), the weight ratio of probucol/PVP was fixed at 1/3 and a surfactant was added at the weight ratio of 0.1, 0.2, 0.3, 0.5 or 1 in a glass vial. The physical mixture (PM) was prepared by mixing for 5 min using vortex mixture. For the preparation of ternary ground mixture (GM), PM was ground in a vibrational rod mill (TI-500ET, CMT Co., Ltd.) for 30 min under liquid N₂ atmosphere. Temperature of the grinding process was controlled at 10 ± 5°C. The grinding cell with capacity of 10 mL and a rod were made of stainless steel.

Powder X-ray diffraction (PXRD) measurement

The powder X-ray diffraction (PXRD) measurements of samples were conducted on a Rigaku (model Miniflex) powder X-ray diffractometer (Tokyo, Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; scanning angle, 2 to 35°; scanning speed, 4°/min.
Solid-state nuclear magnetic resonance (NMR) spectroscopy

All $^{13}$C solid-state NMR spectra were acquired using a JNM-ECA600 NMR spectrometer that has a magnetic field of 14.09 T (JEOL, Tokyo, Japan) and operates at 150 MHz for $^{13}$C. Samples (ca. 100 mg) were placed as powders into 4 mm silicon nitride ($\text{Si}_3\text{N}_4$) rotors. All spectra were acquired using variable amplitude cross-polarization (CP) together with magic angle spinning (MAS) at 15 kHz and a high-power two-pulse phase-modulation $^1$H decoupling. For each spectrum, a total numbers of accumulations (7,000 for ternary systems and 3,000 for an unprocessed sample) were obtained depending on the signal-to-noise ratio required. Pertinent acquisition parameters include relaxation delays of 3 s for all experiments, a cross-polarization (CP) contact time of 5 ms and a $^1$H 90º pulse of 2.7 $\mu$s. The total of data points was 2,048 in each experiment and zero-filled to 8,192 points. All spectra were externally referenced to tetramethylsilane by setting the methine peak of adamantane to 29.5 ppm. The wave separation of $^{13}$C solid-state NMR spectra was performed with computer-fitted curves using the Delta™ NMR Data Processing Software (JEOL, Japan).

Particle size analysis

The volumetric particle size distribution for each suspension was determined by a dynamic light scattering method using Microtrac UPA® or Microtrac FRA® (Nikkiso, Japan). The detection range of UPA is 0.003-6 $\mu$m and for FRA is 0.1-700 $\mu$m. The particle size distribution was measured after sonication of the sample for 2 min.

Stability study in water

The GM was dispersed into distilled water and then sonicated for 2 min to make the suspension. The drug concentration in the suspension was fixed at 0.50 mg/mL. In order to investigate the stability, the suspensions were kept at 25°C. The particle size analysis was conducted at the predetermined time interval of 0 h, 4 h, 1, 3, 7 and 14 days.
Determination of zeta potential

A zeta potential for each suspension in distilled water was determined using ZetaPALS® (Nikkiso, Japan). The average of three tests was reported.

Quantitative determination of the drug as nanoparticles

To determine the quantity of probucol in suspension, 0.5mg/mL of probucol suspensions were prepared, as stated above. Then the suspension was passed through a 0.8 μm membrane filter (nitrocellulose filter, Millipore®). The filtrates obtained were diluted with the HPLC mobile phase solution (acetonitrile/distilled water 185/15 (v/v)). The drug concentration in nanoparticle fractions was determined using HPLC (LC-6A, Shimadzu Co., Japan). The mobile phase was delivered at a flow rate of 1.0 mL/min through a C-18 Inertsil ODS-2 (4.6 mm I.D.×150 mm) at 40 °C and the detection wavelength was 254 nm.
Table 1 Structures and physicochemical properties of probucol.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Physicochemical properties</th>
</tr>
</thead>
</table>
| Probucol | ![Structure Image](image) | • Anticholesterol drug  
• MW: 516.84  
• Water solubility  
  5 ng/mL at 25°C  
• mp 124.5-126 ºC  
  (Form I, stable)  
• mp 116 ºC (Form II) |
Table 2 Structures and physicochemical properties of polymers.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Physicochemical properties</th>
</tr>
</thead>
</table>
| Hypromellose acetate succinate (AQOAT®)                      | ![Structure](image1) | • MW: 17,000-20,000  
• Tg: 119.7ºC  
• soluble in buffer solution at pH > 5.5                                               |
| Methacrylic acid-methyl methacrylate copolymer (1:1) (Eudragit® L100) | ![Structure](image2) | • MW: 135,000  
• Tg: 160ºC  
• Solubility: soluble in intestinal fluid at pH > 6                                                   |
| Polyethylene glycol 4000 (PEG)                              | ![Structure](image3) | • MW: 4,000  
• mp: 50-58 ºC  
• Non-ionic water soluble polymer                                                                  |
| Polyethylene glycol:polyvinyl alcohol (25:75) copolymer (Kollicoat® IR) | ![Structure](image4) | • MW: 45,000  
• mp: 191 ºC  
• Non-ionic water soluble polymer                                                                  |
| Polyvinyl alcohol (PVA)                                     | ![Structure](image5) | • MW: 9,000-10,000  
• mp: 200 ºC  
• Non-ionic water soluble polymer                                                                  |
| Polyvinyl-pyrrolidone (PVP)                                 | ![Structure](image6) | • MW: 10,000  
• mp: soften at 150 ºC  
• Very hygroscopic                                                                                   |
### Table 2 Structures and physicochemical properties of polymers. (cont.)

| Vinylpyrrolidone-vinyl acetate copolymer (60:40) (Plasdone® S630) | ![Chemical Structure] | • MW: 24,000-30,000  
• Tg: 106°C  
• soluble in water |
**Table 3** Structures and physicochemical properties of surfactants.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Physicochemical properties</th>
</tr>
</thead>
</table>
| Polyoxyethylene (20) stearyl ether (Brij® 78)    | ![Polyoxyethylene Structure](image) | • Non-ionic surfactant  
  • MW: 1151.6  
  • CMC = 1 mM  
  • mp: 38 ºC  
  • Oral LD50 in rat  
  2-4g/kg body weight |
| Hexadecyltrimethyl-ammonium bromide (CTAB)       | ![Hexadecyltrimethyl-ammonium Structure](image) | • Cationic surfactant  
  • MW: 364.45  
  • CMC = 0.96 mM  
  • mp: 237 - 243ºC  
  • Oral LD50 in rat  
  410mg/kg |
| Sodium alkyl sulfate                             | C6S ![C6S](image)  
  C8S ![C8S](image)  
  C12S (SDS) ![C12S](image) | • Anionic surfactant  
  Sodium Hexyl Sulfate (C6S)  
  -MW: 204.22  
  -CMC = 420 mM  
  Sodium Octyl Sulfate (C8S)  
  -MW: 232.27  
  -CMC (25 ºC) = 133 mM  
  Sodium Dodecyl Sulfate (C12S, SDS)  
  -MW: 288.38  
  -CMC (25 ºC) = 8.15 mM |
<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Structure</th>
<th>MW</th>
<th>CMC (25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Hexadecyl Sulfate</td>
<td>C16S</td>
<td>344.54</td>
<td>0.5 mM</td>
</tr>
<tr>
<td>Sodium Octadecyl Sulfate</td>
<td>C18S</td>
<td>372.60</td>
<td>0.615 mM</td>
</tr>
</tbody>
</table>
CHAPTER III
RESULTS AND DISCUSSION

A) Effect of polymer species on probucol nanoparticle formation

Particle size measurement

The mean particle size of unprocessed probucol was around 28 µm (Fig. 1). Probucol, water-soluble polymer and SDS were co-ground at weight ratio 1/3/1. The particle size measurement results were summarized in Table 4. After co-grinding with polymer and SDS, probucol nanoparticles with the mean particle size smaller than 1 µm (range from around 90 nm to 600 nm) were obtained when all species of polymer were used. The difference of mean particle size might be due to the unequal degree of strength in intermolecular interaction among the components in solid state.

The appropriate handling of nanoparticle preparation process due to the intrinsic properties of polymer is necessary. AQOAT® and Eudragit® L100 are enteric coating polymer which is soluble in aqueous solution at pH more than 5.5. Due to the melting of the co-ground mixture after grinding at room temperature, cryo-grinding (around -180 ºC) by using liquid nitrogen as a coolant was required when PEG 4000 was used in ternary mixture.

Ternary mixture containing Plasdone® S630, Kollicoat® IR, and PVA could be ground at room temperature. However, the obtained probucol nanoparticles from the co-ground mixtures using Plasdone® S630, Kollicoat® IR, and PVA were larger compared to those obtained from co-ground mixture using PVP.

Taking these results into account, PVP K17 was a promising candidate in probucol nanoparticle formation from ternary co-grinding. The obtained particle size was around 90 nm and water could be used as a dispersing medium. Thus, PVP K17 was used in further investigation of probucol nanoparticle formation with various species of surfactant.
Fig. 1 Particle size distribution of unprocessed probucol in water.
<table>
<thead>
<tr>
<th>Polymer</th>
<th>Grinding time</th>
<th>Mean particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinylpyrrolidone K17</td>
<td>30 min</td>
<td>86 nm</td>
</tr>
<tr>
<td>Plasdone® S630</td>
<td>30 min</td>
<td>192 nm</td>
</tr>
<tr>
<td>AOOAT® (in JP2 fluid)</td>
<td>30 min</td>
<td>294 nm</td>
</tr>
<tr>
<td>Eudragit® L100 (in JP2 fluid)</td>
<td>30 min</td>
<td>376 nm</td>
</tr>
<tr>
<td>Kollicoat® IR</td>
<td>60 min</td>
<td>413 nm</td>
</tr>
<tr>
<td>Polyethylene glycol 4000</td>
<td>60 min</td>
<td>530 nm</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>60 min</td>
<td>592 nm</td>
</tr>
</tbody>
</table>
B) Effect of charge of surfactant on probucol nanoparticle formation

Particle size measurement and percentage of nanoparticle formation

Influence of surfactant charge on probucol nanoparticle formation from ternary co-grinding was investigated. Probucol and PVP were mixed with anionic surfactant (SDS), cationic surfactant (CTAB) or non-ionic surfactant (Brij® 78) at weight ratio of 1/3/0.5 and 1/3/1. Particle size analysis of each sample after dispersing the ground mixture in water was performed and the results are summarized in Table 5. For the same species of surfactant, there was no significant difference in the size of the ground mixture with the weight ratio between 1/3/0.5 and 1/3/1. Probucol nanoparticles with the size of less than 200 nm were produced when any species of surfactants were used.

The percentage of probucol nanoparticle (smaller than 0.8 µm) formation was determined by HPLC (Fig. 2). Co-grinding of probucol, PVP and either ionic or non-ionic surfactant yielded a high percentage of 80-100% of probucol nanoparticle formation. However, comparing to the co-ground mixture obtained from non-ionic surfactant, smaller nanoparticles were obtained from the co-ground mixture using ionic surfactants.

Pandit et al. reported the molecular interaction between PVP and non-ionic surfactants 27,28). The molecular interaction between PVP and CTAB in water was reported by Gharibi et al. 29) and that between PVP and SDS was reported by many researchers 30-32). Speculated from data based on these reports, induced by co-grinding, molecular interaction between PVP and surfactant were also possible to occur in solid state.
**Table 5** Mean particle size of probucol nanoparticles obtained from ternary co-grinding with PVP and different charges of surfactant.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight ratio</th>
<th>Mean particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC:PVP K17:SDS</td>
<td>1:3:0.5</td>
<td>52 ± 24</td>
</tr>
<tr>
<td></td>
<td>1:3:1</td>
<td>81 ± 37</td>
</tr>
<tr>
<td>PBC:PVP K17:Brij®</td>
<td>1:3:0.5</td>
<td>154 ± 63</td>
</tr>
<tr>
<td></td>
<td>1:3:1</td>
<td>188 ± 74</td>
</tr>
<tr>
<td>PBC:PVP K17:CTAB</td>
<td>1:3:0.5</td>
<td>81 ± 33</td>
</tr>
<tr>
<td></td>
<td>1:3:1</td>
<td>82 ± 31</td>
</tr>
</tbody>
</table>
Fig. 2 Percentage of probucol nanoparticle formation from ternary co-grinding with PVP and different charges of surfactant. Weight ratio of probucol/PVP/surfactant was 1/3/0.5 and 1/3/1.
Zeta potential measurement and stability of nanoparticle in water

Zeta potential measurement was conducted in order to understand the surface property of each probucol nanoparticulate system. As shown in Fig. 3, zeta potential of unprocessed probucol was -35.1 mV. The probucol nanoparticles from the ternary ground mixture with SDS, Brij® and CTAB showed the zeta potential of -47.3 mV, -11.2 mV and 47.0 mV, respectively. The difference of the values reflected the charge of the surfactant used. It has been reported that surfactant tends to form a polymer-surfactant complex in an aqueous medium \(^{34-36}\). The polymer-surfactant complex seems to be formed at the solid/liquid surface of probucol nanoparticles.

Stability of the probucol nanoparticles after dispersing the ground mixture in water was investigated at 25°C for 2 weeks and the result was shown in Fig. 4. Agglomeration of the probucol nanoparticle was observed during the first 24 hours, when the ternary ground mixture with SDS and CTAB were used. After that, the particle size became unchanged up to 14 days. In the case of the ground mixture with Brij®, the particle size was within the range of 160-180 nm, which was larger than that of the ground mixtures using ionic surfactants (100-120 nm). Effect of weight ratio of the surfactant on the particle size was not observed. The complex of polymer and surfactant was expected to play an important role for the stabilization of the probucol nanoparticles by preventing the further agglomeration via electrostatic repulsion or steric hindrance. The agglomerations at the first period seem to be due to the insufficient coverage of the complex on the primary particles. Interaction of the particle with the adjacent one would induce the agglomeration. It was speculated that agglomerated particles became stabilized after the surface was fully covered by the complex.
Fig. 3 Zeta potential of ternary ground mixture of probucol/PVP/surfactant.
The weight ratio of ground mixture is 1/3/1. n = 3. Bars represent mean ± SD.
Fig. 4 Stability profiles of ternary ground mixtures of probucol/PVP/surfactant.

■ = probucol/PVP/SDS 1/3/1 weight ratio, □ = probucol/PVP/SDS 1/3/0.5 weight ratio, ● = probucol/PVP/Brij® 1/3/1 weight ratio, ○ = probucol/PVP/Brij® 1/3/0.5 weight ratio, ▲ = probucol/PVP/CTAB 1/3/1 weight ratio, △ = probucol/PVP/CTAB 1/3/0.5 weight ratio.
C) Effect of alkyl chain length of sodium alkyl sulfate on probucol nanoparticle formation

Particle size measurement and percentage of nanoparticle formation

Sodium alkyl sulfates (C6S, C8S, C12S, C16S or C18S) was mixed with probucol/PVP (1/3 w/w) at a weight ratio 0.1, 0.2, 0.3, 0.5 and 1. The particle size analysis of each ternary ground mixture after dispersing in water was performed and the results are demonstrated in Figs. 5-9 and summarized in Table 6. The mean particle size of probucol became smaller consistently with the increase of the surfactant ratio. A single and sharp particle size distribution at nano range was observed when a particular weight ratio of surfactant was reached in each co-ground system. At this weight ratio, the mean particle size of probucol drastically decreased. Additionally, the percentage of probucol nanoparticle formation (Fig. 10) revealed that more than 80% of nanoparticles (smaller than 800 nm) were obtained at this weight ratio. The increase of the weight ratio of a surfactant resulted in the increase of percentage of probucol nanoparticles. However, when the amount of surfactant exceeded this particular weight ratio in each system, the excess amount of the surfactant did not contribute to the further particle size reduction.

At the same weight ratio, more effective probucol nanoparticle formation was observed using the sodium alkyl sulfate with longer alkyl chain. For instance, when C6S was used, the percentage of nanoparticle formation was 1.3% at the weight ratio of 1/3/0.1 then increased up to ca. 100% when the weight ratio became 1/3/1. On the other hand, when the surfactant with a longer alkyl chain C18S was used, the percentage of nanoparticle formation was around 30% at the weight ratio of 1/3/0.1 then increased up to 96-99% at the weight ratio of 1/3/0.3.

Tadeschi et al. \(^{33}\) investigated the interaction between PVP and sodium alkyl sulfate in the solution state by electron paramagnetic resonance spectroscopy. They found that, at the surfactant concentration which was higher than the critical micelle concentration (CMC), the charge density of the surface of micelles increased in the order of C12S>C10S>C8S>C6S. They also reported about the strength of polymer-surfactant association when PVP (\(M_w\ 24000;\ 1\%w/w\)) was introduced into the surfactant solution. The strength of the association increased with the increase of the
alkyl chain length. Additionally, the minimum number of PVP repeating units which required to enclose a single surfactant aggregate was also calculated. The value was around 40 for C10S and around 100 for C12S. In other words, the surfactant with a longer alkyl chain could occupy a PVP molecule by a comparatively smaller amount. These results suggested that a sodium alkyl sulfate with a longer alkyl chain length was more favorable to interact with the nitrogen atom on the pyrrolidone ring of PVP through its negatively charged head groups. Despite the smaller amounts, the surfactant with a longer alkyl chain could induce PVP to properly interact with probucol and produce the nanoparticles.

*Stability of nanoparticles in water*

A stability study of the nanoparticle in an aqueous phase was conducted by monitoring the change of particle size of the suspension (Fig. 11). Nanoparticles of probucol instantly formed after dispersing the ground mixture into water. The mean particle size was still less than 200 nm and was steadily suspended in water without occurrence of precipitation up to 21 days at 25°C. Pongpeerapat et al. 34) suggested that PVP K17 and SDS (C12S) formed a necklace-structure complex covering probucol nanocrystals in the suspension of ternary co-ground mixtures of probucol/PVP K17/SDS at the weight ratio of 1/3/1. Thus in this study, surface covering of PVP-surfactant complexes on probucol nanoparticles was expected to play a role in stabilization of the probucol nanoparticles in the suspension.
Fig. 5 Particle size distribution of probucol nanoparticles obtained from ternary ground mixtures of probucol/PVP/C6S after dispersing into distilled water. The weight ratio of probucol/PVP/C6S is (A) 1/3/0.1, (B) 1/3/0.2, (C) 1/3/0.3, (D) 1/3/0.5 and (E) 1/3/1.
Fig. 6 Particle size distribution of probucol nanoparticles obtained from ternary ground mixtures of probucol/PVP/C8S after dispersing into distilled water. The weight ratio of probucol/PVP/C6S is (A) 1/3/0.1, (B) 1/3/0.2, (C) 1/3/0.3, (D) 1/3/0.5 and (E) 1/3/1.
Fig. 7 Particle size distribution of probucol nanoparticles obtained from ternary ground mixtures of probucol/PVP/C12S after dispersing into distilled water. The weight ratio of probucol/PVP/C6S is (A) 1/3/0.1, (B) 1/3/0.2, (C) 1/3/0.3, (D) 1/3/0.5 and (E) 1/3/1.
Fig. 8 Particle size distribution of probucol nanoparticles obtained from ternary ground mixtures of probucol/PVP/C16S after dispersing into distilled water. The weight ratio of probucol/PVP/C6S is (A) 1/3/0.1, (B) 1/3/0.2, (C) 1/3/0.3, (D) 1/3/0.5 and (E) 1/3/1.
Fig. 9 Particle size distribution of probucol nanoparticles obtained from ternary ground mixtures of probucol/PVP/C18S after dispersing into distilled water. The weight ratio of probucol/PVP/C6S is (A) 1/3/0.1, (B) 1/3/0.2, (C) 1/3/0.3, (D) 1/3/0.5 and (E) 1/3/1.
Table 6 Mean particle size of ternary ground mixtures of probucol/pvp/sodium alkyl sulfate at various weight ratios in distilled water.

<table>
<thead>
<tr>
<th>Weight Ratio</th>
<th>Mean Particle Size&lt;sup&gt;a&lt;/sup&gt; (µm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PBC&lt;sup&gt;b&lt;/sup&gt;/PVP/C6S</td>
</tr>
<tr>
<td>1/3/0.1</td>
<td>19.2 ± 0.1</td>
</tr>
<tr>
<td>1/3/0.2</td>
<td>15.6 ± 0.4</td>
</tr>
<tr>
<td>1/3/0.3</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td>1/3/0.5</td>
<td>10.2 ± 0.0</td>
</tr>
<tr>
<td>1/3/1</td>
<td>0.083 ± 0.003</td>
</tr>
</tbody>
</table>

<sup>a</sup>Results are expressed as mean ± SD (n = 3).

<sup>b</sup>Deviation for probucol.
Fig. 10 Percentage of probucol nanoparticle formation from various species and ratios of sodium alkyl sulfate surfactant determined by HPLC.
Fig. 11 Mean particle size of probucol/PVP/surfactant ternary ground mixtures in distilled water after storage at 25°C for 21 days.

(A) probucol/PVP/C6S, (B) probucol/PVP/C8S, (C) probucol/PVP/C12S, (D) probucol/PVP/C16S and (E) probucol/PVP/C18S. Each point represents the mean ± SD (n = 3).
Powder X-ray diffraction measurement

Solid state properties of ternary co-ground mixtures of probucol, PVP and sodium alkyl sulfate at various weight ratios were investigated using PXRD measurement. The results are shown in Figs. 12-16. In the physical mixtures, the characteristic diffraction peaks of both probucol and a surfactant were observed. In the ground mixtures, the PXRD patterns of each system become halo by grinding. When the weight ratio of a surfactant increased, the characteristic diffraction peaks of the surfactants appeared. Since it was very difficult to identify the characteristic PXRD peaks of probucol in each ground mixture, $^{13}$C solid-state NMR measurements were performed to investigate the molecular state of probucol in the ternary ground mixture.
Fig. 12 PXRD patterns of ternary physical and ground mixtures of probucol/PVP/C6S.

The weight ratio is (P-1, G-1) 1/3/0.1, (P-2, G-2) 1/3/0.2, (P-3, G-3) 1/3/0.3, (P-4, G-4) 1/3/0.5 and (P-5, G-5) 1/3/1.

P = physical mixture, G = ground mixture. ○ = probucol, □ = surfactant.
Fig. 13 PXRD patterns of ternary physical and ground mixtures of probucol/PVP/C8S.

The weight ratio is (P-1, G-1) 1/3/0.1, (P-2, G-2) 1/3/0.2, (P-3, G-3) 1/3/0.3, (P-4, G-4) 1/3/0.5 and (P-5, G-5) 1/3/1.

P = physical mixture, G = ground mixture. ○ = probucol, □ = surfactant.
Fig. 14 PXRD patterns of ternary physical and ground mixtures of probucol/PVP/C12S.

The weight ratio is (P-1, G-1) 1/3/0.1, (P-2, G-2) 1/3/0.2, (P-3, G-3) 1/3/0.3, (P-4, G-4) 1/3/0.5 and (P-5, G-5) 1/3/1.

P = physical mixture, G = ground mixture. ○ = probucol, □ = surfactant.
Fig. 15 PXRD patterns of ternary physical and ground mixtures of probucol/PVP/C16S.

The weight ratio is (P-1, G-1) 1/3/0.1, (P-2, G-2) 1/3/0.2, (P-3, G-3) 1/3/0.3, (P-4, G-4) 1/3/0.5 and (P-5, G-5) 1/3/1.

P = physical mixture, G = ground mixture. ○ = probucol, □ = surfactant.
Fig. 16 PXRD patterns of ternary physical and ground mixtures of probucol/PVP/C18S.

The weight ratio is (P-1, G-1) 1/3/0.1, (P-2, G-2) 1/3/0.2, (P-3, G-3) 1/3/0.3, (P-4, G-4) 1/3/0.5 and (P-5, G-5) 1/3/1.

P = physical mixture, G = ground mixture. ○ = probucol, □ = surfactant.
Solid-state NMR measurement

$^{13}$C solid-state NMR has been used to investigate the molecular state of ternary components in the ground mixture. Molecular interaction among them would be evaluated by the measurement. At first, spectral assignment of the components was performed. The molecular structures and the correspondent $^{13}$C solid-state NMR spectra of probucol, PVP, C6S and C12S are shown in Figs. 17 and 18.

Gerber et al. reported polymorphs of probucol, Form I and Form II $^{35)}$. In probucol Form II (Fig. 17 (B)), the C-S-C-S-C chain is extended and the molecular structure is symmetrical. However, this symmetry is lost in Form I (Fig. 17 (A)) where the torsion angles around C1-S1 and C1-S2 bonds deviate significantly from 180°. Due to the asymmetrical structure of Form I, the carbon 4 existed in the different electromagnetic environments. Hence the splitting of NMR signal at carbon 4 was observed at around 123.9 and 123.1 ppm. On the other hand, signal of carbon 4 of probucol Form II appeared as a single peak, and shifted to the higher field at 118.5 ppm. The unprocessed probucol used in our experiment was confirmed as Form I.
Fig. 17 Molecular structure of probucol and the correspondent $^{13}$C solid-state NMR signals.

The overall NMR spectrum of Form I (A) and Form II (B) Conformation structures of probucol Form I and II were taken from J.J. Gerber $^{35}$. 
Fig. 18 Molecular structures and $^{13}$C solid-state NMR spectra of PVP, C6S and C12S.
Binary grinding of probucol with PVP or probucol with SDS was not enough to reduce the particle size to nano level. The molecular interaction among ternary components was expected to play an important role in particle size reduction. Pongpeerapat proposed the possible intermolecular interaction as shown in Fig. 19. Probucol seemed to interact with PVP through hydrogen bonding between the hydroxyl group of probucol and the carbonyl group of PVP. An electrostatic interaction should exist between the negatively charged head group of SDS and the nitrogen atom on the pyrrolidone ring of PVP.

Figures 20 and 21 show the $^{13}$C solid-state NMR spectra of probucol/PVP/C6S and probucol/PVP/C12S ternary system, respectively. In the $^{13}$C solid-state NMR spectra, interaction between probucol and PVP could be observed at around 170-112 ppm. The interaction between PVP and sodium alkyl sulfate was investigated at around 72-65 ppm. This region showed peak of carbon 1", which was the most proximate to sulfate head group of the surfactant. The signal from the other carbon atoms in an alkyl chain superimposed on the signals originated from probucol and PVP, at around 40-20 ppm. Thus the peaks at this region were very complicate and difficult to identify.

The NMR results showed that probucol existed as Form I polymorph in both co-ground mixture using C6S and C12S. Additionally, in the NMR spectra of ternary ground mixtures with C6S and C12S, peaks of probucol in the range around 170-112 ppm were broad compared with spectrum of their physical mixture. The broad peaks indicated the interaction between probucol and PVP. Moreover, the new peak at around 145 ppm (marked by $\Diamond$) due to the interaction was also observed. Speculated from this result, probucol should exist in the solid state as a core crystal surrounded by amorphous form (indicated by peak broadening) due to the interaction with PVP. In other words, interaction between probucol and PVP occurred only on the surface of probucol crystal.

In probucol/PVP/C6S ternary ground systems (Fig. 20), the carbon 1" peak of C6S shifted from 69.1 ppm to 67.4 ppm at the weight ratio of 1/3/0.3 and to 67.6 ppm at that of 1/3/0.5. At the higher weight ratio of 1/3/1, two peaks were observed. One at 67.7 ppm was the high-field shift due to the molecular interaction between PVP and C6S, another at 70.2 ppm was belonging to the excess amount of C6S crystal.
In probucol/PVP/C12S systems, the similar chemical shifts were observed at the weight ratio of 1/3/0.1 and 1/3/0.2 ((Fig. 21-1). Peaks at the high-field shifted from 69.1 ppm to 67.0 ppm at the weight ratio of 1/3/0.1 and to 67.4 ppm at that of 1/3/0.2 weight ratio. At 1/3/0.3 weight ratio (Fig. 21-2), not only the chemical shift due to the interaction between PVP and C12S, but also the small peak at 69.1 ppm (indicated by arrow) were observed. The latter peak was believed to be originated from the excess amount of C12S crystal. The $^{13}$C solid-state NMR spectrum at the weight ratio of 1/3/1 showed three peaks at 70.4, 69.2 and 67.7 ppm within the range from 70 to 65 ppm. The first two peaks of carbon 1″ of C12S and values of the chemical shift were same as those of the physical mixture. The peak at 67.7 ppm showed the high-field shift from 69.1 ppm due to the molecular interaction between PVP and C12S.
Resonance structure of PVP

Interaction among probucol, PVP and SDS

Electrostatic interaction

Hydrogen bonding

Fig. 19 Proposed intermolecular interaction among probucol, PVP and SDS.

Courtesy of A. Pongpeerapat \textsuperscript{36}. 
Fig. 20 $^{13}$C solid-state NMR spectra of ternary mixture of probucol/PVP/C6S. (A) Physical mixture of probucol/PVP/C6S at 1/3/1 weight ratio. The weight ratio of ground mixture of probucol/PVP/C6S is (B) 1/3/0.3, (C) 1/3/0.5 and (D) 1/3/1.
Fig. 21-1 $^{13}\text{C}$ solid-state NMR spectra of ternary mixture of probucol/PVP/C12S.

(A) Physical mixture of probucol/PVP/C12S at 1/3/1 weight ratio. The weight ratio of ground mixture of probucol/PVP/C12S is (B) 1/3/0.1 and (C) 1/3/0.2.
Fig. 21-2 $^{13}$C solid-state NMR spectra of ternary mixture of probucol/PVP/C12S.

(A) Physical mixture of probucol/PVP/C6S at 1/3/1 weight ratio. The weight ratio of ground mixture of probucol/PVP/C12S is (B) 1/3/0.3 and (C) 1/3/1.
Proposed structure of probucol/PVP/sodium alkyl sulfate

From the NMR measurement results, the structure of probucol/PVP/sodium alkyl sulfate in solid state was speculated and schematically shown in Fig. 22. Probucol existed as a core crystal which surrounded by amorphous probucol due to the interaction with PVP. Simultaneously, PVP also interacted with surfactant and resulted in the chemical shifting of carbon 1" of surfactant. In the state where probucol nanoparticles could not be obtained after dispersing in water (Fig. 22 (A)), the free surfactant crystals were not available. Increasing the weight ratio of surfactant (Fig. 22 (B)) resulted in the existed of the surplus amount of surfactant crystal which assisted in the properly dispersion of probucol nanoparticles after dispersing in water.

The complex formation between PVP and sodium alkyl sulfate on the surface of probucol in water has been reported\(^{34}\). These complexes prevented the agglomeration of probucol nanoparticles. From the results of this study, the structure of probucol/PVP/surfactant after dispersing in water was proposed and shown in Fig. 23. In case of C12S system at the weight ratio lower than 0.3 (Fig. 23 (A)), the amount of surfactant was not enough to form complexes around probucol particles. This resulted in the agglomeration of probucol nanoparticles. Thus, the mean particle size was comparatively large and percentage of nanoparticle formation was low. When the excess amount of surfactant existed (Fig. 23 (B)), the surface coverage of probucol nanoparticles by PVP-C12S complexes resulted in maintaining the nano-sized primary particles in the water.

As described in the section of particle size measurement, the interaction of a sodium alkyl sulfate with a longer alkyl chain with PVP was stronger than that with a shorter one (CAC of PVP-surfactant was lower when the longer alkyl chain surfactant was used). In other words, smaller amount of long alkyl chain sodium alkyl sulfate produced more probucol nanoparticles compared to the shorter one when using at the same weight ratio. That should be the reason why the surfactant with a longer alkyl chain was more effective for probucol nanoparticle formation.
Fig. 22 Proposed structure of probucol/PVP/sodium alkyl sulfate in solid state.

A state when surfactants were low and probucol nanoparticles after dispersing in water were not obtained (A). A state when excess amount of surfactants existed and probucol nanoparticles were obtained after dispersing in water (B).
**Fig. 23 Proposed structure of probucol/PVP/sodium alkyl sulfate after dispersing in water.**

A state when surfactants were low and probucol nanoparticles after dispersing in water were not obtained (A). A state when excess amount of surfactants existed and probucol nanoparticles were obtained after dispersing in water (B).
CHAPTER IV
CONCLUSIONS

Probucol nanoparticles were obtained from ternary co-grinding of probucol, water-soluble polymer and surfactant. However, there was a difference in mean particle size of the nanoparticles obtained from utilizing different polymers and surfactants. In order to gain the insight information of probucol nanoparticle formation, the investigation was conducted.

Chapter III-A

After co-grinding with 7 polymers and SDS, probucol nanoparticles with the mean particle size smaller than 1 µm were obtained when all species of polymer were used. The difference of mean particle size might be due to the unequal degree of strength in intermolecular interaction among the components in solid state. Among the polymer used in this study PVP exhibited a superior particle size reduction over other polymers after co-grinding.

Chapter III-B

Probucol nanoparticles below 200nm were obtained when the co-grinding with PVP and different charges of surfactant were conducted. Zeta potential results showed that PVP-surfactant complexes existed on the surface of probucol nanocrystals. The complex played an important role for stabilization of the nanoparticles in water either by electrostatic repulsion or steric hindrance.

Chapter III-C

The effect of alkyl chain length of sodium alkyl sulfates on probucol nanoparticle formation was investigated. Particle size distribution and HPLC results indicated that the anionic surfactant with longer alkyl chain contributed more effectively to the probucol nanoparticle formation when the weight ratio of the ternary components was same. The amount of probucol nanoparticles increased with increase of that of the surfactant. The surfactant content-dependent intermolecular interaction among the ternary components was effectively investigated by $^{13}$C solid-state NMR spectrometry.
These results suggested that the excess amount of surfactant was required for the stabilizing nanoparticles after dispersing in water.

It was estimated that many of newly developed drug compounds are poorly water-soluble or insoluble. Preparation of drug as nanoparticles is expected to be a promising method to improve the solubility profiles of poorly water-soluble drug and hence improve the bioavailability of such drugs. These findings brought about the important aspects for the formulation optimization of drug/polymer/surfactant ternary co-grinding system. This comprehensive knowledge on specific drug-additive interactions will pave the way forward a more scientific basis for additives selection.
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REFERENCES


LIST OF PUBLICATION

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LIST OF COMMITTEE

This thesis, conducted for the Degree of Doctoral of Philosophy (Pharmaceutical Sciences) was examined by the following committee, authorized by the Graduate School of Pharmaceutical Sciences, Chiba University, Japan.

Professor Yasushi Arano, Ph.D., Chairman
(Graduate School of Pharmaceutical Sciences, Chiba University)

Professor Hiromitsu Takayama, Ph.D.
(Graduate School of Pharmaceutical Sciences, Chiba University)

Professor Atsushi Nishida, Ph.D.
(Graduate School of Pharmaceutical Sciences, Chiba University)