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Nano-pulverization of poorly water soluble compounds with low melting points by a rotation/revolution pulverizer

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We report a method for pulverizing poorly water soluble compounds with low melting points to nanoparticles without producing an amorphous phase using a rotation/revolution pulverizer. Fenofibrate, flurbiprofen, and probucol were used as crystalline model compounds. They were suspended in a methylcellulose aqueous solution and pulverized with zirconia balls by the rotation/revolution pulverizer. Beeswax, an amorphous compound, was also examined to investigate whether nano-pulverization of a compound with a low melting point was possible. Beeswax was suspended in ethyl alcohol cooled with liquid nitrogen and pulverized with zirconia balls by the rotation/revolution pulverizer. By optimizing the pulverization parameters, nanoparticles ($D_{50} < 0.15$ μm) of the crystalline compounds were obtained with narrow particle size distributions at a rotation/revolution speed of 1000 rpm and a rotation/revolution ratio of 1.0 when the vessel was 0 °C. Amorphous fenofibrate and flurbiprofen were not detected by differential scanning calorimetry or powder X-ray diffraction, whereas small amounts of amorphous probucol were detected. Beeswax was pulverized to nanoparticles ($D_{50} = 0.14$ μm) with ethyl alcohol cooled with liquid nitrogen. Fine nanoparticles of these poorly water soluble compounds with low melting points were obtained by controlling the rotation/revolution speed and reducing the vessel temperature.

1. Introduction

Advances in combinatorial chemistry and high-throughput screening have resulted in an increase in the number of drug candidates with high pharmacological activity in pharmaceutical development. However, most of them have poor water solubility and their bioavailability is low (Lipinski 2000, 2002; Lipinski et al. 2001). Pulverization is one method for enhancing solubility (Filippos et al. 2007). Reducing the particle size of a compound increases its surface area and its dissolution rate according to the Nernst–Brunner and Levich modifications of the Noyes–Whitney equation for dissolution (Dressman et al. 1998; Horter and Dressman 2001). Solubility is also enhanced by reducing the particle size to the order of nanometers according to the Ostwald–Freundlich equation (Kipp 2004). Nano-pulverization has the advantages of increasing bioavailability and reducing fed/fasted effects of drugs and it also has the potential to realize intravenous sustained release (Jinno et al. 2006; Rabinow 2004). However, conventional pulverizers generate heat during pulverization, which causes heat-labile compounds to degrade and eventually become amorphous at high temperatures (Saleki-Gerhardt et al. 1994).

We previously used a rotation/revolution pulverizer to nano-pulverize phenytoin (Takatsuka et al. 2009). Nanosizing by a rotation/revolution pulverizer is a form of wet bead milling. Phenytoin was pulverized at high energy by zirconia balls that were accelerated by the rotation and revolution of the vessel. The rotation/revolution pulverizer is superior to conventional bead mills as it has shorter pulverizing times (a few minutes) and it can use fewer zirconia balls to pulverize phenytoin to the order of nanometers without producing the amorphous phase.

In this study, fenofibrate, flurbiprofen and probucol were selected as poorly water soluble crystalline compounds with a low melting point (mp) (82, 115, and 125 °C, respectively), which is much lower than that (295 °C) of phenytoin. Beeswax, which is an amorphous compound and has a mp of 60 °C, was also examined to investigate whether nano-pulverization of a compound with a very low mp was possible. This paper describes the method used to pulverize poorly water soluble compounds with a low mp to nanoparticles using the rotation/revolution pulverizer.

2. Investigations, results and discussion

2.1. Pulverization of fenofibrate, flurbiprofen, and probucol under the optimum conditions for phenytoin by the rotation/revolution pulverizer

In preliminary tests, phenytoin was pulverized to nanoparticles under the optimum conditions by the rotation/revolution pulverizer (Table 1). Fenofibrate, flurbiprofen, and probucol were pulverized under the same conditions as phenytoin. However, their pulverized particles aggregated so that a well-dispersed nanosuspension could not be obtained. This is because zirconia balls with high collision energies seemed to activate the particle surfaces during pulverization (Inkyo and Tahara 2004; Inkyo et al. 2006). Tween 80 or SDS was added to 0.3% (w/v) MC aqueous solution to prevent nanoparticle aggregation. Fenofi-

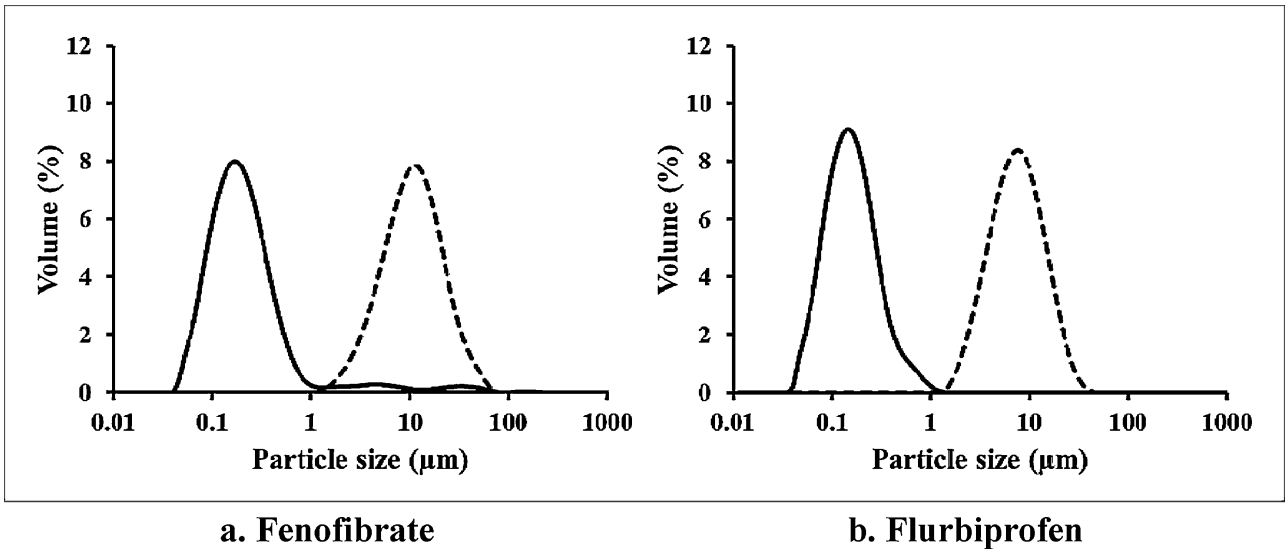

Fig. 1: Particle size distribution of original compounds (- - - -) and compounds pulverized by the rotation/revolution pulverizer under the optimum pulverizing conditions for phenytoin (—)

Table 1: Optimum pulverizing conditions for phenytoin by the rotation/revolution pulverizer

Parameter		
Revolution speed (rpm)		2000
Pulverizing time (min)		2
Volume of 0.3% MC solution (ml)		0.5
Zirconia balls	Diameter (mm)	0.1
	Mass (g)	2.5
Temperature during pulverizing (°C)		ca. 30

brate and flurbiprofen could be pulverized to nanoparticles with 0.3% (w/v) MC aqueous solution containing 0.1% (v/v) Tween 80 (Fig. 1). However, probucol nanoparticles could not be obtained by pulverizing with a 0.3% (w/v) MC aqueous solution containing 0.1% (v/v) Tween 80. Probucol nanoparticles were obtained when probucol was pulverized with a 0.3% (w/v) MC aqueous solution containing 1% (w/v) SDS (Fig. 2). SDS was suitable for preparing stable nanoparticles as in the case of drug/PVP/SDS (Pongpeerapat et. al. 2004). A freeze-dried sample of the pulverized compound was analyzed by PXRD to evaluate the crystallinity of the compound (Fig. 3). As shown in Fig. 3, the PXRD pattern of the original compound contains several intense peaks, whereas all the pulverized compounds exhibit a halo diffraction pattern. This indicates that amorphous phases of each compound were produced and that the optimum pulverizing conditions for phenytoin were not suitable for fenofibrate, flurbiprofen, and probucol. The low mp compounds are considered to have a lower crystal binding energy than phenytoin, which has a higher mp. The accumulation of heat generated by collisions of the zirconia balls, the crystals of the compound, and the vessel wall increases the temperature of the vessel and its contents, which promotes the production of the amorphous phase. When the zirconia balls have a high collision energy and the compound has a low mp, the heat generated by collisions may melt the crystals and produce the amorphous phase. It is thus important to control the collision energy to suppress heat generation during pulverization. The collision energy was estimated according to Eq. (1) (Kano et al. 2000):

$$Ew = \sum_{i=1}^{n} \frac{1}{2w} m v_i^2 \quad (1)$$

where E_w is the collision energy, w is the mass of the compound, m is the mass of a zirconia ball, v_i is the speed of a zirconia ball relative to the compound particles, and n is the number of collisions between the zirconia ball and the particles of the compound. Equation (1) shows that the collision energy decreases with decreasing speed of the zirconia balls relative to the compound particles. Therefore, the collision energy at a rotation/revolution speed of 1000 rpm is about one fourth

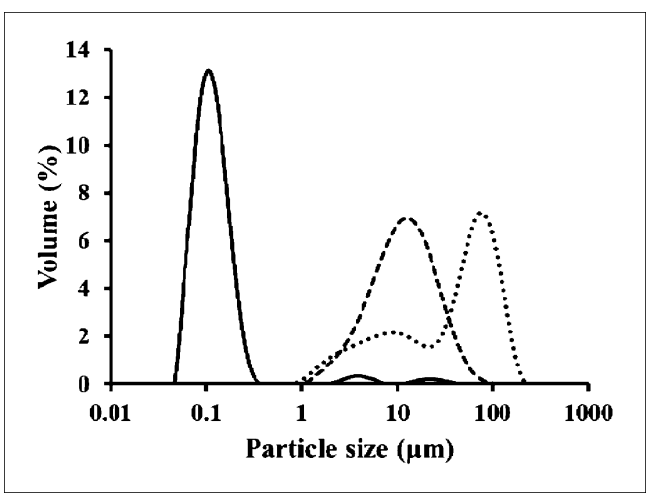

Fig. 2: Particle size distribution of the original probucol (- - - -), probucol pulverized using 0.3% (w/v) MC aqueous solution containing 0.1% (v/v) Tween 80 (···), and probucol pulverized with 0.3% MC (w/v) aqueous solution containing 1% (w/v) SDS (—). Probucol was pulverized by the rotation/revolution pulverizer using the optimum pulverizing conditions for phenytoin

Table 2: Mild pulverizing conditions for fenofibrate, flurbiprofen, and probucol by the rotation/revolution pulverizer

Parameter		
Revolution speed (rpm)		1000
Pulverizing time (min)		4
Volume of 0.3% MC solution (ml)		0.5
Zirconia ball	Diameter (mm)	0.1
	Mass (g)	2.5
Temperature during pulverizing (°C)		ca. 10

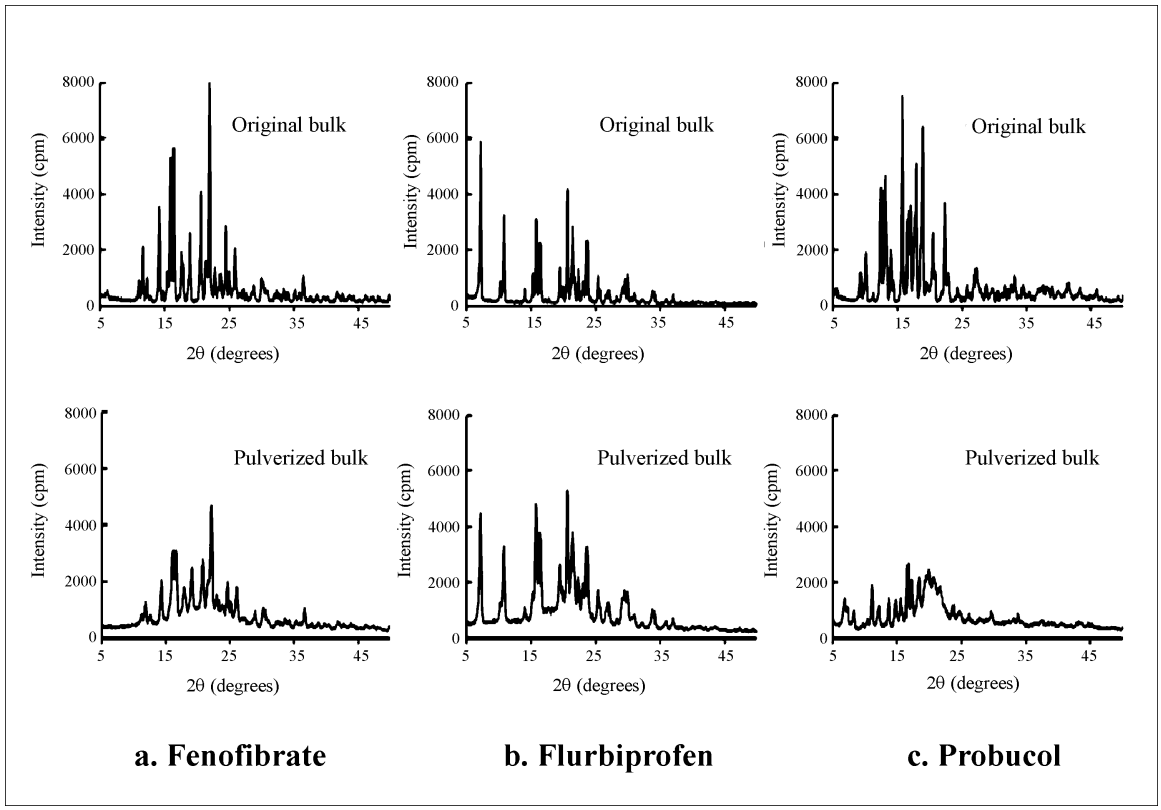

Fig. 3: PXRD patterns of the original compounds (upper) and compounds pulverized by the rotation/revolution pulverizer under the optimum pulverizing conditions for phenytoin (bottom)

that at 2000 rpm. The increase in the temperature of the vessel and its content can be reduced by cooling the vessel. The rotation/revolution speed was reduced from 2000 to 1000 rpm to reduce the collision energy and the vessel was cooled to about 0 °C.

2.2. Pulverization of fenofibrate, flurbiprofen, and probucol under mild conditions by the rotation/revolution pulverizer

Fenofibrate, flurbiprofen, and probucol could be pulverized to nanoparticles under the optimum pulverizing conditions for phenytoin, but amorphous phases of these compounds were produced. The pulverizing conditions under which these compounds could be pulverized to nanoparticles without producing their amorphous phases were investigated. Table 2 shows the pulverizing conditions for fenofibrate, flurbiprofen, and probucol that reduce the collision energy to suppress heat generation. The diameter and the amount of zirconia balls and the volume of the MC solution were the same as above. The pulverizing time was varied from 1 to 8 min. Fenofibrate were not sufficiently pulverized after pulverizing times in the range 1 to 2 min, and the particle size of pulverized fenofibrate did not vary for pulverizinig times exceeding 4 min, as shown in Fig. 4. Fenofibrate was well pulverized after 4 min and D_{50} and D_{90} were 0.129 and 0.313 μm, respectively. The conditions in Table 2 that lowered the collision energy and suppressed heat generation are referred to as the mild conditions in this study. Fenofibrate and flurbiprofen were pulverized to nanoparticles by the rotation/revolution pulverizer under these mild conditions (Figs. 5a and b). Whereas a well-dispersed nanosuspension could not be obtained without Tween 80 for the optimum pulverizing conditions for phenytoin, it could be obtained without Tween 80 under the mild conditions. D_{50} and D_{90} of the pulverized particles under the optimum pulverizing conditions for phenytoin were also smaller than under the mild conditions (Table 3). Obviously, activation of the particle surfaces was suppressed by the lower collision energy of the mild conditions (Inkyo et al. 2006). This demonstrates that activation of the particle surfaces and particle aggregation can be prevented by controlling the collision energy. However, probucol was not fully pulverized to nanoparticles under the mild conditions. Probucol is hydrophobic and has a lower wettability than fenofibrate and flurbiprofen. Probucol was pulverized at ambient temperature with a 0.3% (w/v) MC solution containing 1% (w/v) SDS to improve its wettability (Fig. 5c). Freeze-dried samples of pulverized fenofibrate, flurbiprofen, and probucol were analyzed by PXRD and DSC to investigate the crystallinity of the pulverized particles (Figs. 6 and 7). As shown in Fig. 6, the PXRD patterns of all three compounds did not change on pulverization under the mild conditions. Halo diffraction patterns were not obtained for pulverized fenofibrate and flurbiprofen (Figs. 6a and b). Exothermal DSC peaks, which indicate the presence of

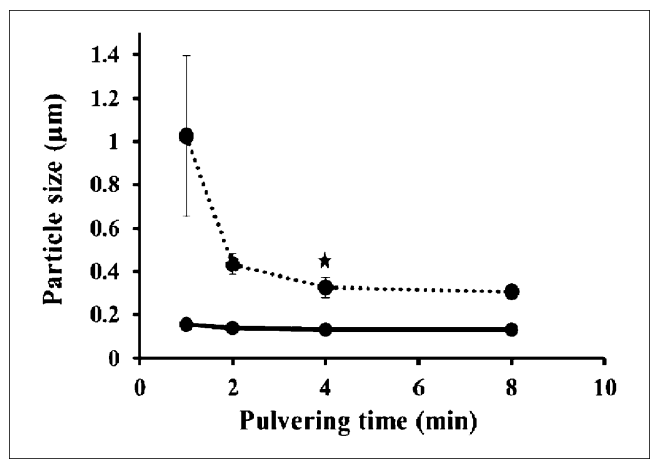

Fig. 4: Effect of pulverizing time on D_{50} (—) and D_{90} (- - - -) of pulverized fenofibrate (rotation/revolution speed: 1000 rpm)

Table 3: D_{50} and D_{90} of compounds pulverized under the optimum pulverizing conditions of phenytoin and mild pulverizing conditions by the rotation/revolution pulverizer ($n = 3$)

Compound	Particle size			
	Optimum conditions for phenytoin		Mild conditions	
	D_{50} (μm)	D_{90} (μm)	D_{50} (μm)	D_{90} (μm)
Fenofibrate	0.146 ± 0.005	0.435 ± 0.078	0.126 ± 0.002	0.314 ± 0.095
Flurbiprofen	0.163 ± 0.009	0.407 ± 0.033	0.130 ± 0.002	0.312 ± 0.025
Probucol	0.125 ± 0.001	0.259 ± 0.017	0.108 ± 0.004	0.186 ± 0.004

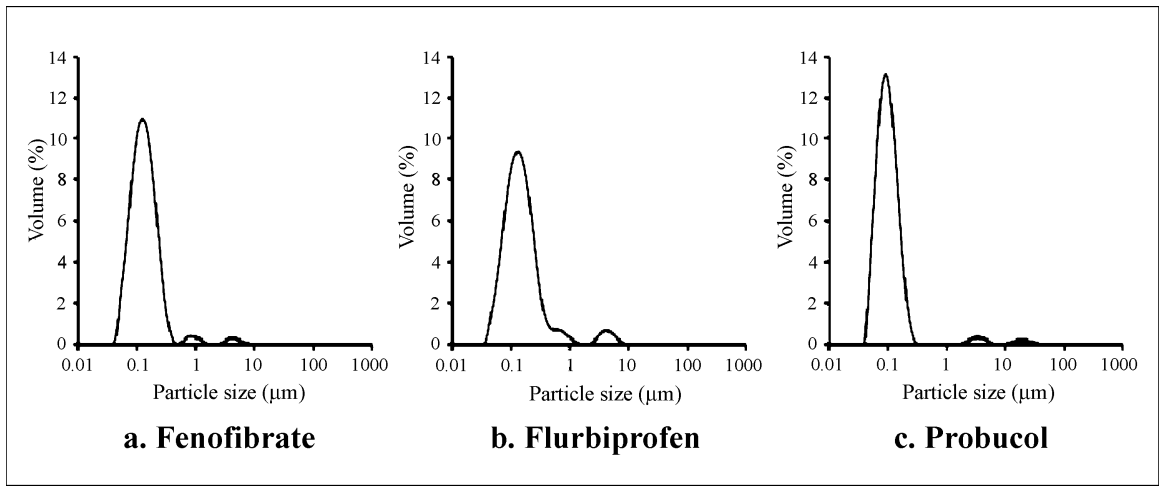

Fig. 5: Particle size distribution of compounds pulverized by the rotation/revolution pulverizer under the mild pulverizing conditions

the amorphous phase, were not observed for pulverized fenofibrate and flurbiprofen (Figs. 7a and b). A halo diffraction pattern (Fig. 6c) and an exothermal peak at 122–123 °C (Fig. 7c) were observed for pulverized probucol, which indicate the presence of the amorphous phase.

2.3. Pulverizing beeswax

Nano-pulverization by the rotation/revolution pulverizer could be applied to powders of poorly water soluble compounds with a low mp. We also investigated nano-pulverization by the rotation/revolution pulverizer of a waxy compound with a low mp, beeswax. Beeswax could not be pulverized under the optimum pulverizing conditions for phenytoin. The form of beeswax did not change and some zirconia balls attached to the surface of the beeswax after the process. It is thought that beeswax could not be pulverized because it is soft and extensible at ambient temperature. Beeswax was therefore frozen using liquid nitrogen to reduce its extensibility during pulverizing. It was pulverized by applying three processes: dry milling, wet milling, and dispersion. Dry milling was performed to homogenize the particle size of beeswax pounded in a mortar and pestle. Wet milling was carried out to pulverize the nanoparticles. Ethyl alcohol was used as a solvent to pulverize beeswax below 0 °C. An optimized three-stage dispersion process was conducted to prevent nanoparticle aggregation (Table 4). By milling with liquid nitrogen as reported in a previous study fine nanoparticle were not obtained (Niwa et al. 2010). On the other hand, the rotation/revolution pulverizer with liquid nitrogen could pulverize beeswax to nanoparticles ($D_{50} = 0.139$ μm, $D_{90} = 0.937$ μm) under the optimum pulverizing conditions for beeswax, as shown in Fig. 8.

2.4. Conclusion

The proposed method for pulverizing using a rotation/revolution pulverizer is highly suitable for reducing the particle sizes of poorly water soluble compounds with a low mp to the order

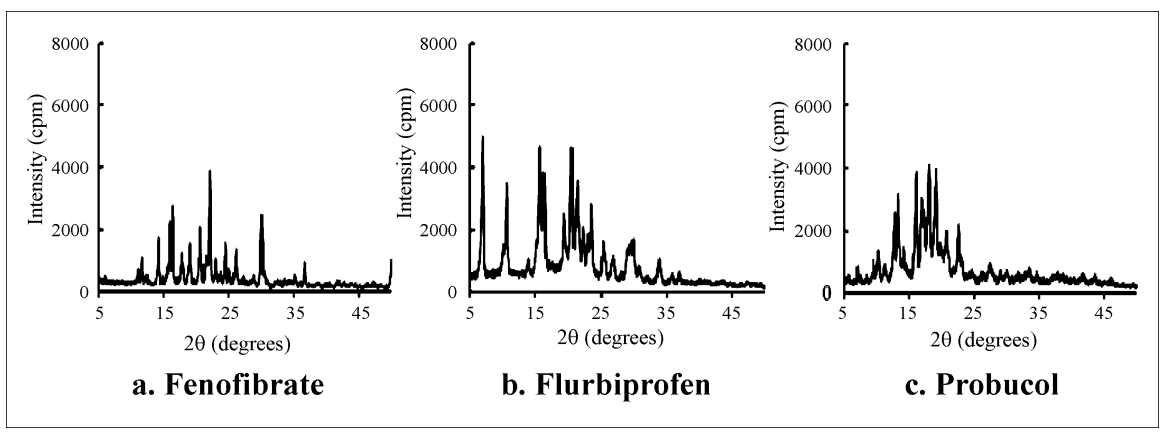

Fig. 6: PXRD patterns of the compounds pulverized by the rotation/revolution pulverizer under the mild pulverizing conditions

Table 4: Optimum pulverizing conditions for beeswax in the three processes by the rotation/revolution pulverizer

Parameter		Dry milling	Wet milling	Dispersion
Revolution speed (rpm)		2000	2000	1000
Pulverizing time (min)		2	4	10
Volume of ethanol (ml)			1	10
Zirconia balls	Diameter (mm)	0.1	0.1	0.05
	Mass (g)	2.5	2.5	10
Liquid nitrogen (ml)		80	160	Not added

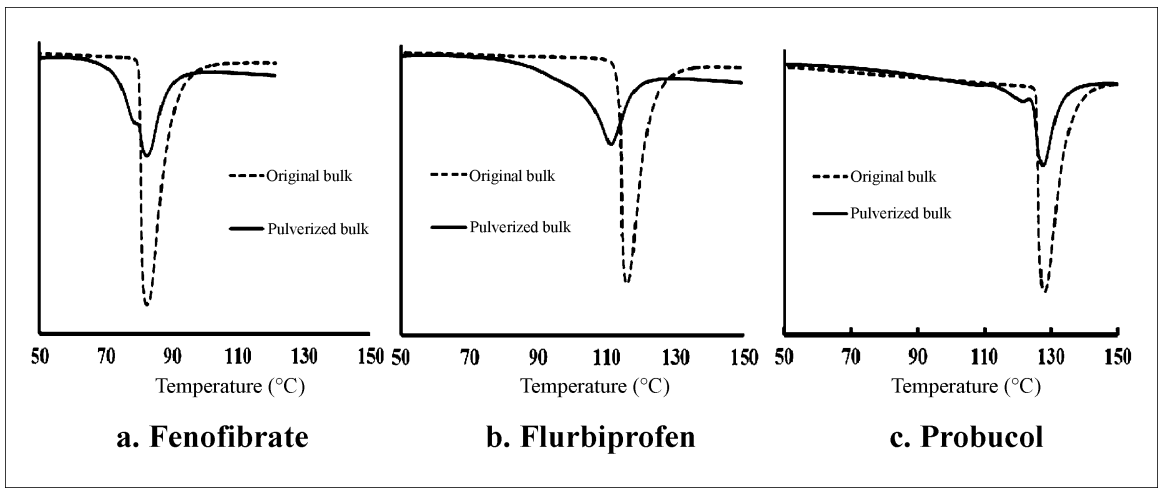

Fig. 7: DSC thermographs of original compounds (- - - -) and compounds pulverized by the rotation/revolution pulverizer under the mild pulverizing conditions (—)

of nanometers. Nanoparticles of crystalline compounds were obtained with a narrow particle size distribution under mild conditions. The amorphous phases of fenofibrate and flurbiprofen were not detected by DSC and PXRD analysis, whereas a small amount of amorphous probucol was detected. It is important to control the collision energy and the temperature during pulverization and to choose an appropriate solvent to obtain nanoparticles without producing the amorphous phase of the compound. Beeswax, a semi-solid and waxy compound, could also be pulverized using the rotation/revolution pulverizer with ethyl alcohol cooled in liquid nitrogen. Using the rotation/revolution pulverizer with liquid nitrogen has the potential to pulverize to nanoparticles both chemicals and biomaterials, such as phospholipids, proteins, enzymes, and antibodies. Nanopulverization of biomaterials will enable the development of novel methods of drug delivery.

3. Experimental

3.1. Materials

Fenofibrate, probucol, and beeswax were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan) and flurbiprofen was purchased from Sigma-Aldrich Corp. (Missouri, US). Methylcellulose (MC) (Metolose SM-4000) and polyvinylpyrrolidone K30 (PVP) were purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and Wako Pure Chemical Industries Co., Ltd., respectively. Tween 80 and sodium lauryl sulfate (SDS) were purchased from Wako Pure Chemical Industries Co., Ltd. and Nacalai Tesque Inc. (Kyoto, Japan), respectively. Zirconia (zirconium oxide) balls with diameters of 0.1, 0.05, and 0.03 mm were purchased from Nikkato Co., Ltd. (Osaka, Japan). All other reagents were analytical-grade commercial products.

3.2. Methods

3.2.1. Preparation of nanosuspensions of crystalline compounds (fenofibrate, flurbiprofen, and probucol)

Compound (100 mg) was weighed into the zirconia vessel of the rotation/revolution pulverizer (NP-100, Thinky Corp., Tokyo, Japan); 2.5 g of 0.1-diameter-mm zirconia balls were placed into the vessel and the appropriate volume of 0.3% (w/v) MC aqueous solution was added. The following four steps were used to prepare suspensions: (1) pulverization at 1000 rpm for 4 min with 0.5 mL of 0.3% (w/v) MC aqueous solution; (2) pulverization at 400 rpm for 1 min after adding 9.5 mL of 0.3% (w/v) MC aqueous solution (total volume: 10 mL); (3) separation of 0.1-mm-diameter zirconia balls from the suspension; (4) pulverization at 2000 rpm for 3 min after adding 2.5 g of 0.03-mm-diameter zirconia balls to the suspension.

3.2.2. Preparation of nanosuspensions of an amorphous compound (beeswax)

Pellets of beeswax (5-mm-diameter) were frozen with liquid nitrogen and pounded into a powder using a mortar and pestle. Pounded beeswax powder (100 mg) and 30 mg of PVP were added to the zirconia vessel of the rotation/revolution pulverizer, then 5 g of 0.1-mm-diameter zirconia balls and liquid nitrogen were added to the vessel. The following five steps were used to prepared suspensions of beeswax: (1) (dry milling) pulverization at 2000 rpm for 2 min with about 40 ml of liquid nitrogen, which

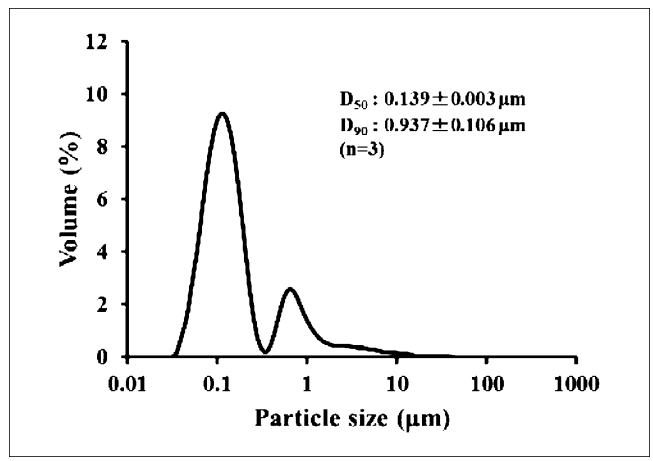

Fig. 8: Particle size distribution of beeswax pulverized by the rotation/revolution pulverizer under the optimum pulverizing conditions for beeswax

was added every minute; (2) (wet milling) pulverization at 2000 rpm for 4 min after adding 1 ml of ethyl alcohol and 40 ml of liquid nitrogen, liquid nitrogen was added every minute; (3) dispersion at 400 rpm for 1 min after adding 9 ml of ethyl alcohol (total volume: 10 ml); (4) separation of 0.1-mm-diameter zirconia balls from the suspension; (5) dispersion at 1000 rpm for 10 min after adding 10 g of 0.05-mm-diameter zirconia balls to the suspension.

3.3. Particle size distribution

The suspensions of the pulverized compounds were diluted threefold with a saturated solution of each compound and sonicated for 10 min. The samples were analyzed by a laser diffractometer (Mastersizer 2000, Malvern Instruments, UK) with a small-volume dispersing unit (Hydro 2000 μP, Malvern Instruments). The size distribution was expressed in terms of the diameters at 50% (D_{50}) and 90% (D_{90}) of the population distribution.

3.4. Powder X-ray diffraction (PXRD)

PXRD patterns were obtained using RINT (Rigaku Corp., Japan) with Cu radiation generated at 40 mA and 40 kV. Data were obtained from 3 to 50° (2θ) at a scanning speed of 5 °/min.

3.5. Differential scanning calorimetry (DSC)

DSC was performed using a DTG-60 (Shimadzu Corporation, Kyoto, Japan). DSC thermograms were obtained in an open aluminum pan using about 8 mg of the sample and about 7 mg of alumina as the standard material. The sample was heated from 50 to 120 or 150 °C at a heating rate of 5 °C/min with a 50 mL/min argon purge.

References

Dressman JB, Amidon GL, Reppas C, Shah VP (1998) Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm Res 15: 11–22.

Filippos K, Santipharp P, Yunhui W (2007) Nanosizing – Oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev 59: 631–644.

Horter D, Dressman JB (2001) Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. Adv Drug Deliv Rev 46: 75–87.

Inkyo M, Tahara T (2004) Dispersion of agglomerated nanoparticles by fine beads mill. J Soc Powder Technol Japan 41: 578–585.

Inkyo M, Tahara T, Iwaki T, Iskandar F, Hogan CJ Jr, Okuyama K (2006) Experimental investigation of nanoparticle dispersion by beads milling with centrifugal bead separation. J Colloid Interface Sci 304: 535–540.

Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, Toguchi H, Liversidge GG, Higaki K, Kimura T (2006) Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. J Control Release 111: 56-64.

Kano J, Miyazaki M, Saito F (2000) Ball mill simulation and powder characteristics of ground talc in various types of mill. Adv Powder Tech 11: 333–342.

Kipp JE (2004) The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int J Pharm 284: 109–122.

Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 44: 235–249.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46: 3–26.

Lipinski CA (2002) Poor aqueous solubility – an industry wide problem in drug discovery. Am Pharm Rev 5: 82–85.

Niwa T, Nakanishi Y, Danjo K (2010) One-step preparation of pharmaceutical nanocrystals using ultra cryo-milling technique in liquid nitrogen. Eur J Pharm Sci 41: 78–85.

Pongpeerapat A, Itoh K, Tozuka Y, Moribe K, Oguchi T, Yamamoto K (2004) Formulation and stability of drug nanoparticles obtained from drug/PVP/SDS ternary ground mixture. J Drug Del Sci Tech 14: 441–447.

Rabinow BE (2004) Nanosuspensions in drug delivery. Nat Rev Drug Discov 3: 785–796.

Saleki-Gerhardt A, Ahlneck C, Zografi G (1994) Assessment of disorder in crystalline solids. Int J Pharm 101: 237–247.

Takatsuka T, Endo T, Jianguo Y, Yuminoki K, Hashimoto N (2009) Nanosizing of poorly water soluble compounds using rotation/revolution mixer. Chem Pharm Bull 57: 1061–1067.