

Homogeneous nanoparticles to enhance the efficiency of hydrophobic drugs characterized by solid-state NMR

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Supporting Information

***In vitro* drug release studies:** The *in vitro* drug release test was demonstrated by the JP XV round basket method at 50 rpm and 37°C, using a dissolution tester (NTRVS6P; Toyama-Sangyo Co. Ltd., Japan). JP the 1st fluid (pH 1.2) and JP the 2nd fluid (pH 6.8) were used as the dissolution media. The samples containing approximately 100 mg of PBC were weighed and placed in the dissolution test basket. Five milliliters of the solution was withdrawn, and an equal volume of fresh medium was immediately added at designed time intervals (5, 10, 15, 30, 60, 90, 120, 180 and 240 min). The concentration of PBC was determined by HPLC as described later section.

HPLC conditions: The samples were filtered through a 0.8 µm membrane filter (Millipore Corp., Tokyo, Japan) and then diluted with acetonitrile. The concentration in nanoparticle fractions was determined by HPLC (LC-6A, Shimadzu Corp., Kyoto, Japan). The mobile phase (acetonitrile/water 95/5, v/v) was delivered at a flow rate of 1.0 ml/min through a C-18 column (Inertsil ODS-3, 5µm, 4.6 mm i.d. × 150 mm, GL Sciences Inc., Tokyo, Japan) at ambient temperature and the detection wavelength was 254 nm.

Particle size distribution of SD particles: Particle size was measured by SEM image of particles and image processing software (NS2K-pro, Nanosystem Corp., Kyoto, Japan). The particle size distribution data and the diameters of d₁₀, d₅₀ and d₉₀ are reported based on volume.

Moisture content determination: Samples of ~ 15 mg were placed in aluminum pans and heated at a rate of 5 K/min in a thermogravimetric analyzer (TG8120, Rigaku Corp., Tokyo) linked to a data station (Thermo plus 2, Rigaku Corp., Tokyo). % moisture was calculated as the weight loss between room temperature and 150°C where the profiles leveled off.

PBC content determination: Samples of 10 mg were dissolved with THF, and then diluted with HPLC mobile phase. Probucol in the samples were determined by HPLC.

Powder properties of SD particles: The angles of repose of the spray dried particles were measured with a turntable apparatus (Tsutsui Rikagaku Kikai, Tokyo). Compressibility index (CI) was calculated using the following equation: $CI = (D_f - D_0)/D_f \times 100$ where D_0 is powder density before tapping and D_f is powder density after tapping infinitely. Powder densities were measured using a tapping density analyzer (Tapdenser KYT-1000[®] Seishin Enterprise, Tokyo) containing a 10 mL cylinder.

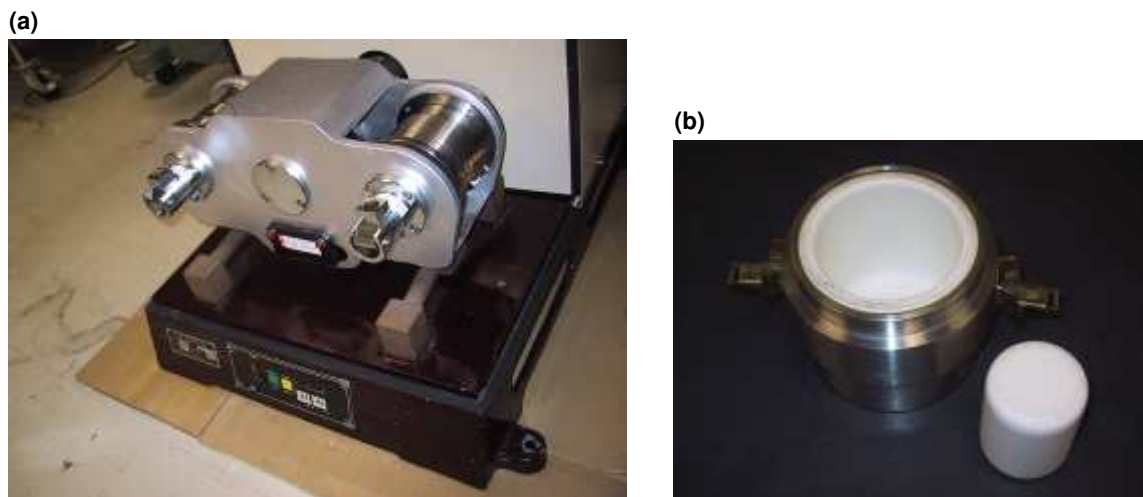
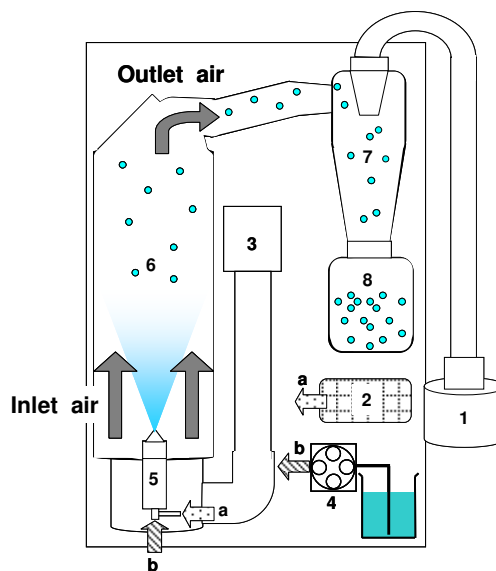


Figure S1. Photographs of vibrating rod mill for cogrinding of drug and additives: (a) apparatus; (b) sample pot and rod.

Figure S2. Schematic diagram of the spray dryer used: (1) blower; (2) air compressor; (3) heater; (4) peristaltic pump; (5) atomizer; (a) compressed air; (b) feed spray drying suspension; (6) drying chamber; (7) cyclone; (8) dry product collector.



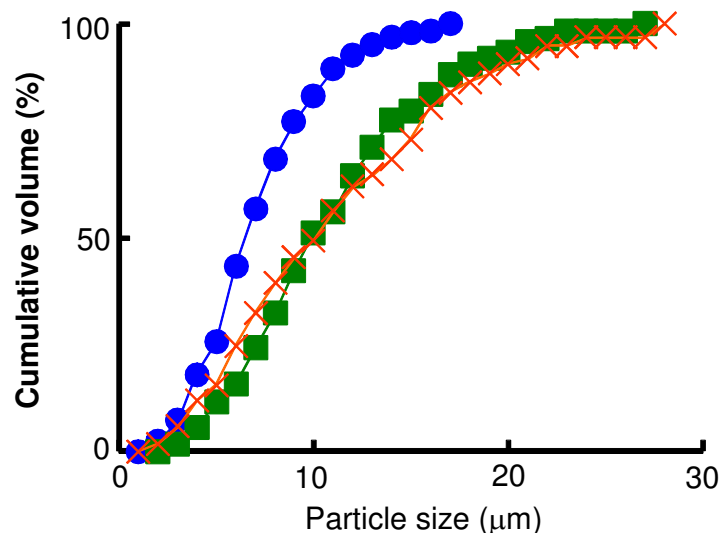


Figure S3. Particle size distribution of the spray dried particles; SD75 (●), SD90 (■), and SD (×). Particle size distribution increased with increased outlet air temperature.

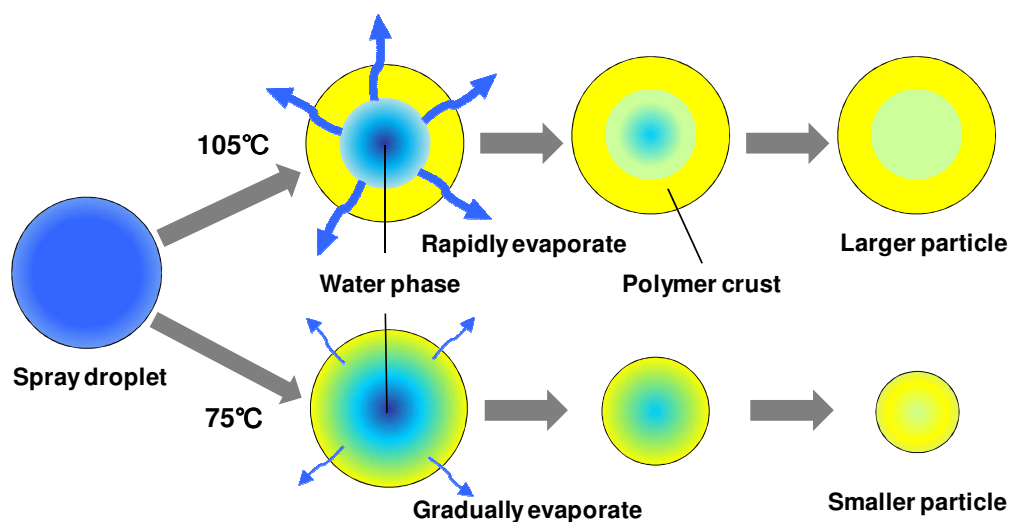


Figure S4. Schematic diagram of speculated process in the formation of spray-dried particles. Inner water phase gradually evaporated at lower temperatures providing smaller particles, whereas a crust was formed on the outer surface of the spray droplets at higher temperatures, resulting in larger particle size.

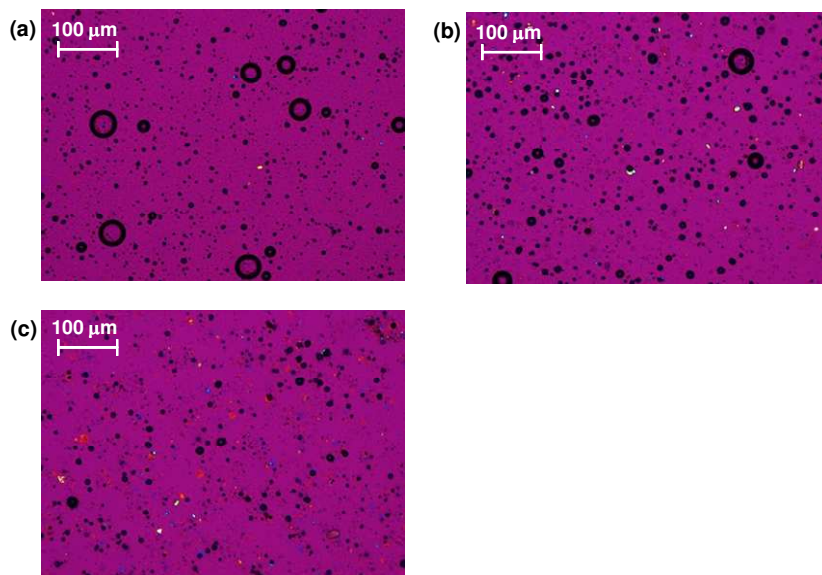


Figure S5. Polarized photographs of the particles produced by spray drying with different preparing temperatures; (a) SD75; (b) SD90; and (c) SD105. Crystalline PBC was hardly observed in the SD75, whereas crystalline components were slightly increased in SD 90 and 105 which were prepared at higher temperature.

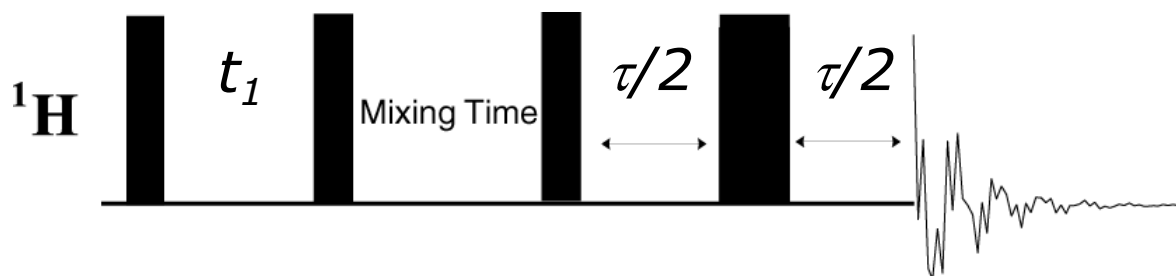


Figure S6. A pulse sequence used for recording NOESY type $^1\text{H}/^1\text{H}$ correlation spectrum under 18 kHz MAS. The spinning frequency of the sample was synchronized with the mixing time. The spin echo was applied before the signal acquisition to filter out short T_2 peaks of spectrum, which are broader components in the spectrum.

Figure S7. 2D $^1\text{H}/^1\text{H}$ NOESY spectra of (a) PBC-I and (b) PBC-II were obtained using a pulse sequence given in **Figure S6** with $\tau = 1$ ms and a mixing time of 11.11 ms under 18 kHz MAS at 27°C in a Bruker 900 MHz NMR spectrometer; 16 scans and 62 t_1 increments were used with the synchronization of the MAS spinning speed and the mixing time. (c) 2D HETCOR spectrum of PBC-II that correlates the chemical shifts of ^{13}C and ^1H of PBC-I under 18 kHz MAS at 27°C. 0.5 ms CP contact time for polarization transfer, 196 scans and 48 t_1 increment were used. States *et al* method was applied to achieve quadruple acquisition in the second dimension.

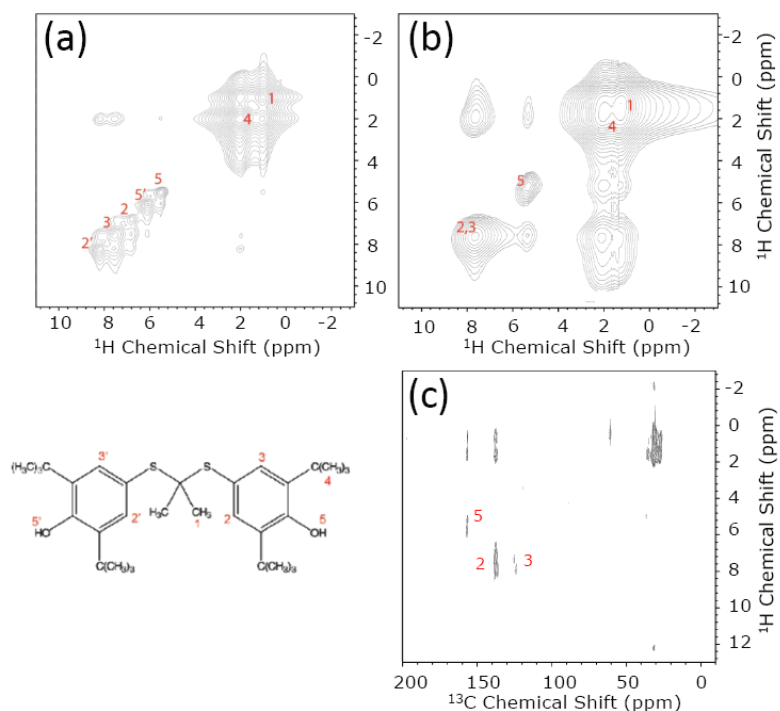


Figure S8. ^1H chemical shift spectra of PBC-I (bottom) and PBC-II (top) were recorded using a solid-echo pulse sequence with a 1 ms refocusing time under 18 kHz MAS at 27°C in a Bruker 900 MHz NMR spectrometer. The molecular structure of PBC and the labeling of proton atoms are shown.

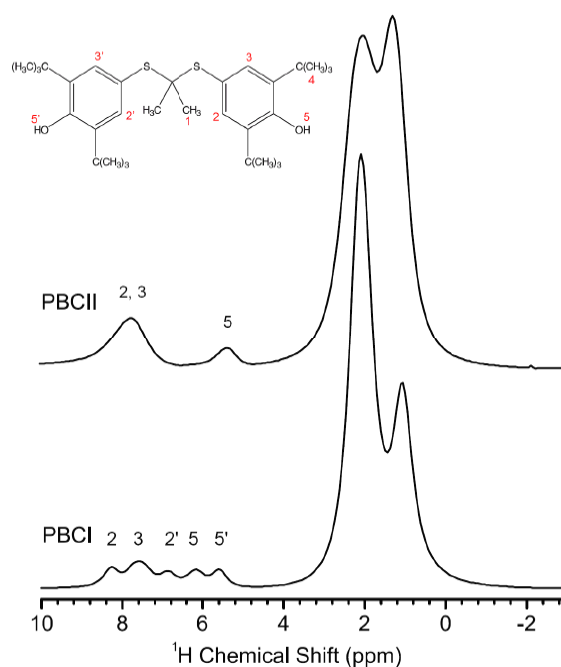


Figure S9. ^{13}C chemical shift spectra of PBC-I (bottom) and PBC-II (top) were obtained using a ramped-CP pulse sequence under 18 kHz MAS at 27°C in a Bruker 900 MHz NMR spectrometer. 1.2 CP contact time was used and a 50 kHz rf power of TPPM decoupling was used during acquisition. The molecular structure of PBC and the labeling of carbon atoms are shown.

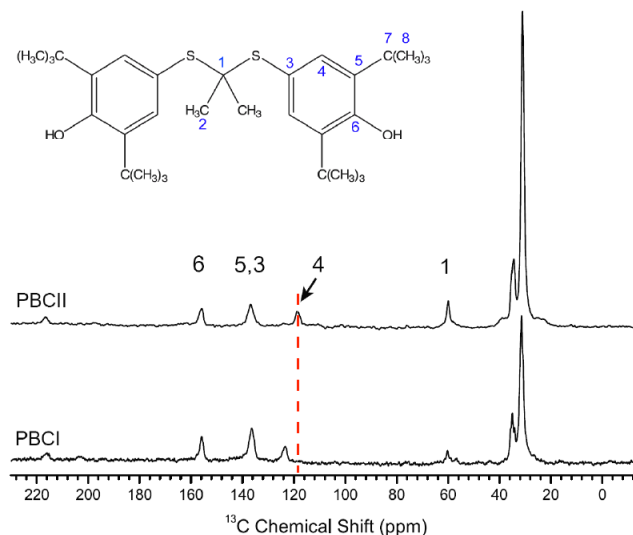


Figure S10. 2D $^1\text{H}/^1\text{H}$ NOESY spectrum of a ground mixture of MMC, SDS and PBC prepared without spray drying was obtained using a pulse sequence given in **Figure S7** with $\tau = 1$ ms and a mixing time of 11.11 ms under 18 kHz MAS at 27°C in a Bruker 900 MHz NMR spectrometer; 16 scans and 62 t_1 increments were used with the synchronization of the MAS spinning speed and the mixing time.

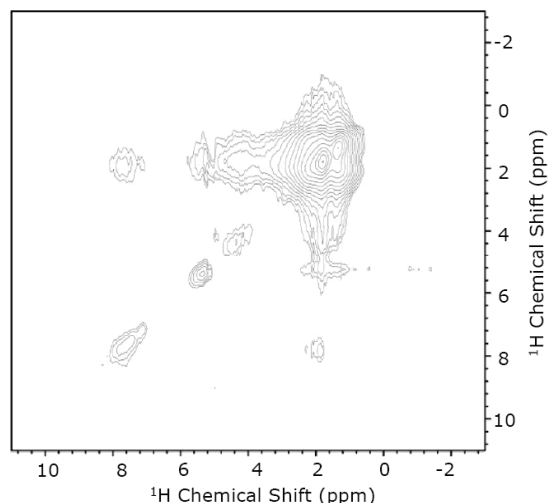


Table S1. Formulation of spraying suspension.

Ingredients	Weight (g)	Content* (%)
PBC/MMC/SDS (1:5:1) GM	7.0	
Triethyl citrate	2.5	50.0
1.7% Ammonium hydroxide	1.7	
Glycerol monostearate	0.3	5.0
Polysorbate 80	0.1	40.0 (on GMS)
Water	37.9	
Total	49.5	

*Content was based on dry polymer.

Solid content :23.0%, Polymer content: 7.45%, Drug loaded: 14%.

Table S2. Spray drying conditions.

Parameters	Value
Inlet air temperature (°C)	85, 100, 120
Outlet air temperature (°C)	75, 90, 105
Blow flow (m ³ /min)	0.6
Atomizing pressure (kg/cm ³)	0.8
Spraying rate (g/min)	2.0
Spraying time (min)	20

Table S3. Particle size distribution of the spray dried particles prepared with different temperatures. Mean diameter value indicates the mean S.D. (n = 4000-7000). The mean diameters of particles were similar among SD75, SD90 and SD105. In contrast, each of the d₅₀ was increased with increased outlet air temperature.

	SD75	SD90	SD120
Mean diameter (μm)	3.3 ± 2.0	3.4 ± 2.5	3.2 ± 2.5
d ₁₀ (μm)	3.8	4.5	4.5
d ₅₀ (μm)	6.7	9.5	10.7
d ₉₀ (μm)	11.2	17.9	19.5

Table S4. PBC and moisture content of the SD particles.

	SD75	SD90	SD105
PBC content (%)	10.4 ± 0.6	11.2 ± 0.4	11.7 ± 0.3
Moisture content (%)	5.1 ± 0.2	5.3 ± 0.3	4.9 ± 0.3

Each value indicates the mean ± S.D. (n = 3).

Table S5. Fluidity of the particles prepared by SD. (n = 3-4).

	SD75	SD90	SD105
Angle of repose (deg.)	52	54	55
Bulk density (ρ _B , g/mL)	0.29	0.23	0.23
Tapped density (ρ _T , g/mL)	0.54	0.48	0.45
Compressibility index ((ρ _T -ρ _B)/ρ _T ×100, %)	46.58	51.36	48.89
Hausner ratio (ρ _T /ρ _B)	1.88	2.06	1.96
Yield (%)	48.2	56.8	53.8