Pharm Res (2011) 28:2339–2349
DOI 10.1007/s11095-011-0465-y

RESEARCH PAPER

Design of Dry Nanosuspension with Highly Spontaneous Dispersible Characteristics to Develop Solubilized Formulation for Poorly Water-Soluble Drugs

Toshiyuki Niwa • Satoru Miura • Kazumi Danjo

Received: 29 March 2011 / Accepted: 27 April 2011 / Published online: 28 May 2011

ABSTRACT

Purpose The powderization of the aqueous nanosuspension of a poorly water-soluble drug, which was prepared by wet-milling technique developed by authors, was investigated to apply to the development of solid dosage forms.

Methods Drug particles were suspended and milled in the aqueous medium using the oscillating beads-milling apparatus. The recovered nanosuspension was spray-dried 1) with no additive or 2) with co-dissolving mannitol as a nanoparticle carrier. As a control, the solution of the drug and additives with the same formulation as nanosuspension was also spray-dried.

Results SEM observation and X-ray powder diffraction analysis revealed that the dried products from suspension formed a spherical particle with single-micron diameter, which was composed of thousands of nano-sized crystalline drug fragment. It was also found that the dried products from suspension could be spontaneously redispersed in water, transforming into nanosuspension with the original size distribution. Such dried powder with high dispersibility was named "dry nanosuspension." The dry nanosuspension had immediate release behaviors in gastrointestinal buffered media, whereas the dried product from solution showed the poor dispersion and dissolution properties even if same content of additives were loaded.

Conclusions The present technique with combination of wet nano-milling and spray-drying processes would be a novel approach to develop the pharmaceutical products with poorly water-soluble and oral-absorbable drugs.

T. Niwa (✉) • S. Miura • K. Danjo
Department of Industrial Pharmacy, Faculty of Pharmacy
Meijo University
150 Yagotoyama, Tempaku
Nagoya 468-8503, Japan
e-mail: niwat@meijo-u.ac.jp

KEY WORDS dry nanosuspension · nano-milling · redispersion · solubilization · spray drying

INTRODUCTION

Over last 20 years, the number of poorly water-soluble drugs has steadily increased in the pharmaceutical market. Also in development stages, progress in discovery technologies, e.g. combinatorial chemistry and high throughput screening methods, leads to a great number of newly discovered compounds that have poor water solubility. As a result, it is reported that 40% of commercial drugs or 60% of promising compounds, so-called "candidates," in the pipelines are categorized as poorly soluble (1–5). These compounds are mainly classified as biopharmaceutical classification system (BCS) class II for which their oral absorption is limited by their rate of dissolution (6). To improve their dissolution behaviors, formulation researchers have already developed diversified solubilization technologies. These include solid dispersions prepared by spray-drying (7,8), freeze-drying (9) or hot melt extrusion (10); complex formation with water-soluble excipients (11,12); particle size reduction/milling (13,14); self-emulsifying drug-delivery systems (SEDDS) (15,16); and so on.

During the discovery and preclinical development stages, a discovery project team often faces the problem of poor oral absorption of the candidates caused by their poor aqueous solubility. The team sometimes pushes back to medicinal chemists to retry chemical modification of lead compounds to seek more hydrophilic ones. However, it is quite difficult to introduce the hydrophilic moiety in the candidate while keeping its high biological potency. The discovery members make the decision to cease such lead optimization activities and select only one champion

candidate with multi-dimensional excellent profiles except solubility, and then put their strong hope on formulation technologies enhancing the dissolution of the poorly water-soluble candidate. For formulation researchers, it is a challenging task to draw up the technical road map to widely cover from preclinical to clinical and commercial development at discovery stage. Despite the availability of a multitude of above solubilization technologies, a universal approach that can be also applied to exploratory research activities during the early development stages, i.e. discovery and preclinical stages, has not been found at present because all technologies described above usually require a specific machine and/or large amount of the active compound to investigate the feasibility. In order to overcome such technical limitations, the authors have developed the universal nano-milling technique in aqueous phase (17). The resultant suspension with nanometer-sized drug particles, namely nanosuspension, is a typical oral dosage form for animal studies. Because the nanosuspension containing 10-mg to 10-g orders of drugs could be prepared using conventional and popular equipments for laboratory use, this approach should become an easily accessible solubilization tool fitted for *in vivo* screening and profiling researches. The suspension dosage is beneficial for animal studies due to quantitative and easy administration but is not appropriate to clinical and commercial use, since its physical and chemical stability are difficult to assure for a long period. The powderization of nanosuspension would be strongly required to expand the application of the current milling technology into late development and commercial stages.

Considering the development of major solid dosage forms, i.e. tablets and capsules, applicable to the clinical and commercial use, the drying of the nanosuspension prepared using a previously established milling apparatus was investigated. The spray-drying method was selected to remove the water in this research because of its popular and productive process from an industrial perspective (18–20). The final goal of this research is to develop the nanomilling and consecutive spray-drying method as novel solubilization technique. Phenytoin was used as a poorly water-soluble model drug. The recovered spray-dried powder should be redispersed immediately in aqueous fluids such as digestive juices and reconstructed into the original milled particles with equivalent size in order to attain their expected pharmaceutical performances. The dried powder which can be spontaneously transformed to nanodispersed suspension is named "dry nanosuspension" in this paper. The operational procedure and formulation were established and optimized to obtain dry nanosuspension with high dispersibility. As a control, the solution dissolving phenytoin and additives with the same composition as the nanosuspension were also spray-dried. Both products prepared from nanosuspension and solution were comparatively evaluated from physicochemical and pharmaceutical viewpoints to clarify the advantages of dry nanosuspension.

MATERIALS AND METHODS

Chemicals

Phenytoin and sodium lauryl sulfate were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). D-mannitol (Mannit P) and polyvinylpyrrolidone (Kollidone 30) were provided by Mitsubishi Shoji Foodtech Co., Ltd. (Tokyo, Japan) and BASF Japan Ltd. (Tokyo, Japan), respectively. Phenytoin, polyvinylpyrrolidone and sodium lauryl sulfate were abbreviated to Phe, PVP and SLS in this report, respectively. All other chemicals and solvents were of analytical reagent grade, and deionized-distilled water was used throughout the study.

Manufacturing Instruments

In this study the oscillating beads-milling apparatus (Multi-Beads Shocker, Yasui Kikai Co., Osaka, Japan) was used for wet-milling of drug particles in aqueous phase. The schematic diagram and operational procedure of wet-milling have been reported in our previous paper (17). Zirconia (zirconium oxide) beads with 0.3 mm in diameter (YZB03) and glass beads with 0.5 mm in diameter (YGBLA-05) were provided by Yasui Kikai Co. The spray-dryer with two-fluid nozzle (SD-1000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) was used to produce the dried powder from the nanosuspension.

Preparation of Milled Particles and Their Spray-Dried Product

The wet-milling process using the oscillating beads-milling apparatus was established in our previous study (17). The drug nanosuspension was prepared according to the standard condition as follows: 1.2 g of phenytoin and 60 g of zirconia beads with 0.3 mmϕ were weighed into a conical tube with 50-mL capacity and suspended in 15 mL of aqueous co-dispersing medium of 0.5% PVP and 0.1% SLS. The tube was put into the holder of the apparatus and oscillated at 2700 rpm for 12 min while cooling the holder at 0°C by circulating a refrigerant. A couple of sets of suspension in the tube were simultaneously milled to obtain sufficient amount of nanosuspension for the next recovery and drying processes.

Then, the nanosuspension was separated from beads and spray-dried to recover the product as a dried powder as schematically illustrated in Fig. 1. Two to four sets of

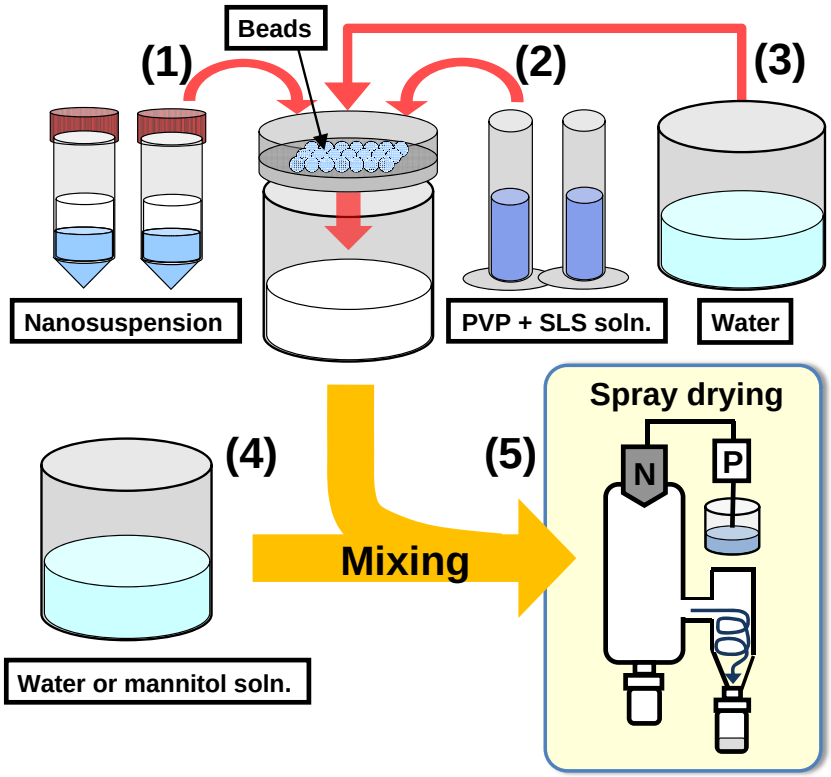

Fig. 1 Schematic diagram of recovery and spray-drying processes of nanosuspension. Preparation steps: (1) remove the beads from nanosuspension using a sieve, (2) wash the beads using dispersing medium, (3) wash the beads using water, (4) dilute the nanosuspension with water or aqueous solution of mannitol, (5) spray-dry the nanosuspension.

nanosuspension obtained through the above milling process were passed together through a sieve with a 150 μm-opening to remove the beads from the suspension. The beads were washed with same volume of the fresh dispersing medium and then washed with appropriate volume of water to withdraw the clung drug particles and dispersing agents. The recovered suspension was diluted with water or aqueous solution of mannitol to adjust the solid content to 1 w/v%. Then, the sample solution was spray-dried through a two-fluid nozzle. The driving conditions were fitted with 130°C inlet air temperature, 70°C outlet air temperature, 10 mL/min spray rate, 150 kPa atomizing air pressure, and 0.65 m^3/min flow air volume. The resultant spray-dried powders were collected and stored in glass vials in a desiccator at room temperature before the physicochemical and pharmaceutical assessments.

The milled suspension was subjected to the spray-drying process in the following two manners: 1) no additive or 2) mannitol co-formulated in the suspension. The spray-drying process or its product from nanosuspension with no additive was shortly described as "Sus-SD," and that with mannitol as "Sus-SD+M." As a control, the spray-dried product with no additive was also prepared from solution, in which phenytoin was entirely dissolved by adjusting the basic pH using ammonium solution and abbreviated to "Sol-SD." The formulations of spray suspension and solution were shown in Table I. The concentration of solid component was set to 1 w/v% in all three spray samples. Both spray fluids of Sus-SD and Sol-SD have the same formulation, but the only difference is whether phenytoin is suspended or dissolved in them. The spray suspension in Sus-SD+M contains same amount of mannitol as phenytoin. The weight ratio of dispersing agents to drug is identical in all three samples (Phe:PVP:SLS = 40:5:1).

Particle Size Distribution (PSD) and Morphology

The particle size distributions of the original bulk particles and wet-milled particles in the prepared nanosuspension were measured by a laser diffraction scattering method using the diffractometer (LMS-30, Seishin Enterprise Co. Ltd., Tokyo, Japan). The original particles dispersed in 0.5% of PVP aqueous solution or small aliquot of nanosuspension were diluted with water appropriately in the batch-type cell. In addition, PSDs of the spray-dried particles were also measured with the same laser diffractometer in dry condition. The dried particles were dispersed into dry air at fixed air pressure of 0.4 MPa. The obtained data were plotted as frequency distribution as

Table I Formulation of Nanosuspension and Solution Supplied to Spray-Drying Process

		State of spray samples		
		Nanosuspension		Solution
Materials	unit	Sus-SD[a]	Sus-SD+M[a]	Sol-SD[b]
Phenytoin	mg	4800	2400	4800
Mannitol	mg		2400	
PVP	mg	600	300	600
SLS	mg	120	60	120
28% ammonium solution[c]	mL			59.1
Water	mL	ad.	ad.	ad.
Total[d]	mL	552	516	552

[a] Symbols of "Sus-SD" and "Sus-SD+M" indicate the spray-dried particles prepared from the nanosuspension with Phe:PVP:SLS = 40:5:1 and Phe:Man:PVP:SLS = 40:40:5:1, respectively.

[b] Symbol of "Sol-SD" indicates the spray-dried particles prepared from the solution with Phe:PVP:SLS = 40:5:1.

[c] The aqueous ammonium solution was formulated to dissolve phenytoin in the spray solution

[d] The spray suspension and solution were diluted with water to set to 1.0 w/v% of the solid concentration.

a function of logarithmic particle size. The diameters at the 10%, 50%, and 90% of the cumulative volume distribution, named D10, D50, and D90, respectively, were represented as size distribution. All measurements of PSD were repeated in triplicate, and the average sizes were reported. The morphology of spray-dried particles was observed under a scanning electron microscope (SEM, JSM-6060, JEOL Ltd., Tokyo, Japan) after coating using a platinum sputtering equipment (JFC-1600, JEOL Ltd.).

Physical Stability of Nanosuspensions

The nanosuspension prepared at a concentration of 80 mg/mL was filled in a glass vial with 10-mL capacity and stored at 4°C and room temperature while avoiding exposure to the light. A small aliquot of suspension was periodically withdrawn from the bottom of whole suspension to measure the PSD until 3 weeks of storage. Apart from vials for PSD evaluation, the appearance was visually observed at same time point to check the settling behavior.

Crystalline Analysis

The crystalline properties of the spray-dried particles were evaluated by X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC). XRPD analysis was conducted using a Geiger-Flex difractometer (RAD-2VC, Rigaku Co., Tokyo, Japan) with CuKα_1 radiation and a Ni filter at a voltage of 30 kV and a current of 20 mA. Samples were scanned over 2θ range of 5–40° at a rate of 5°/min. DSC measurement was performed using DSC instrument (DSC-60, Shimadzu Co. Ltd., Kyoto, Japan). Around 5 mg of dried particles was placed in an open aluminum pan. The heating program was carried out using a modulated setting at 10°C/min over 30–310°C under nitrogen gas flow.

Self-Dispersibility of Spray-Dried Product in Aqueous Phase

One hundred mg of each spray-dried powder was put into 10 mL of water in the glass tube and voltexed for 30 s to make the suspension. The resultant suspension was not sonicated in this study. The PSD of redispersed particles was measured and compared to that of the original nanosuspension to assess the self dispersing property (self-dispersibility).

In Vitro Dissolution Behavior

The dissolution experiments from the spray-dried particles were examined with a dissolution tester (NTR-3000, Toyama Sangyo Co. Ltd., Osaka, Japan) using the paddle method according to Japanese Pharmacopeia fifteenth edition (JP15). Sample powders corresponding to 10 mg of phenytoin were weighed and placed into 900 mL of the dissolution media with holding temperature at 37±0.5°C. The rotation speed of the paddle was set to 50 rpm in this experiment. The dissolution tests of each sample were performed in both the first fluid (pH1.2) and second fluid (pH6.8) defined in the dissolution test of JP15 to estimate the dissolution in the gastrointestinal juice. Aliquots (1 mL) of the solution were taken after 1, 3, 5, 7, 10, 15, 20, 30, 40, 50, 60, 90, 120, 180 min and filtrated through a hydrophilic PTFE membrane filter with 0.20-μm pore size (Dismic-13HP, Advantec Toyo, Tokyo, Japan). The separation efficiency of the filter between solid and dissolved components was validated by comparing to the concentration of supernatant after ultracentrifugation (40,000 rpm, 60 min). Subsequently, the same volume of fresh medium was added to the dissolution vessel. The filtrates were diluted in methanol to the appropriate concentration. The quantity of phenytoin dissolved was assayed spectrophotometrically at 258 nm by HPLC (LC-10, Shimadzu Co. Ltd.) equipped with a reverse-phase column (Inertsil ODS-3, 5 μm, 4.6×150 mm, GL Sciences). The phenytoin peak was eluted around 10 min when running mobile phase (10 mM Na_2HPO_4 solution : acetonitrile, 70:30 v/v) at 1.0 mL/min. Dissolution profiles for phenytoin original powder and each physical mixture with the same composition as spray-dried products were also studied as references. The dissolution test was done in triplicates for each sample, and the mean release percentage was plotted.

RESULTS AND DISCUSSION

Concept of Dry Nanosuspension

In our previous report, simple and universal wet-milling techniques to prepare oral nanosuspension of poorly water-soluble compounds were developed to support the discovery and preclinical studies using animals (17). In addition to our research, many other nano-sizing techniques, including wet-milling (21–25) and high-pressure homogenization (26,27), have been also reported to improve dissolution and absorption performances in the gastrointestinal tract (28). Although the wet-milling technique in aqueous phase is well known as the most effective and successful way to produce the nanometer-sized drug particles, the resultant milled suspension has to be additionally dried to be applied to development of the tablet and capsule, which are major oral dosage forms. Furthermore, once the prepared dry powder is immersed in the aqueous media, it should be discrete and redispersed into the original milled particles with equivalent size, hence restructuring the nanosuspension, in order to attain their expected pharmaceutical performances. The dried particles which can be spontaneously transformed to nanodispersed suspension in aqueous fluid such as digestive juices are called "dry nanosuspension" in this paper.

According to previous reports, freeze-drying is the most popular way to obtain the powder from the milled suspension (29–31). Actually, this method based on freeze and sublimation phenomena of water tends to produce the porous and bulky powder, which is highly advantageous to be soaked up by and dispersed in water. However, freeze-drying is not suitable for industrial production because lyophilization is quite a time-consuming process. Therefore, the spray-drying method, which is much more productive, was applied to eliminate the water in this study. Technically, transformation of nanosuspension into solid product has been achieved using spray-drying operation as reported by other researchers (25,32). Often, large amounts of additives such as dispersing agents or hydrophilic additives are added to the suspension prior to the drying step in order to acquire adequate dispersion in water. In this study the composition of these "dispersion promoters," e.g., surfactants or hydrophilic polymers, was investigated to be minimized to avoid their toxicity attributing to such surface active action, especially at high dosing.

Finalization of Driving Conditions for Oscillating Beads-Milling Apparatus

Almost all driving conditions using an oscillating beads-milling apparatus have been optimized in our previous research (17). Additionally, the effect of the material of beads on milling efficiency was investigated in this study. Sixty g of zirconia beads with 0.3 mmϕ were replaced by 24 g of glass beads with 0.5 mmϕ (both beads had the same packing volume) as a low cost option, and the machine was driven in the standard condition. The progress of size reduction of phenytoin particles during the milling process was exhibited side-by-side in Fig. 2. It was found that the particle sizes were drastically reduced after only 3 min driving in both beads, but the milling efficiency of zirconia beads (Fig. 2B) was much higher than that of glass beads (Fig. 2A). When using zirconia beads, the whole distribution was entirely shifted to submicron range (<1 μm) at 12 min, and further size reduction was still continued. In contrast, the micronization was reached to a plateau around 30 min, and the particles with single-micron size still remained at 39 min using glass beads. D10, D50, D90 values of the 12-min milled particles by zirconia beads and those of the 39-min milled particles by glass beads were 0.141, 0.292, 0.550 μm, and 0.313, 0.637, 1.163 μm, respectively. It was concluded that the heavier beads might accelerate the pulverization in the current oscillating motion. The suspension prepared by 12-min milling using 0.3 mmϕ zirconia beads was subjected to the spray-drying process.

Physical Stability of Nanosuspension During Storage

The intact nanosuspension prepared in the standard condition was transferred to a glass vial and stored in ambient and refrigerated conditions. The representative diameters of the suspended particles were plotted as a function of storage time as shown in Fig. 3. It was found that the size distribution of suspended particles kept unchanged for 3 weeks in refrigerated storage (Fig. 3a), whereas nanoparticles tended to grow gradually under room temperature, resulting in 20% increase of D50 value after 21 days (Fig. 3-b). The size enlargement of suspended particles would be attributed to Ostwald ripening, in which smaller particles are consumed in growth of larger particles originating from the enhanced solubility arising from the higher curvature of smaller particles, as described by the Ostwald-Freundlich equation (33). It is assumed that the cooling of suspension could effectively slow down the ripening, possibly stopping the particle growth for a short time.

As a result of ripening, the particle settling was visually confirmed, which is expressed as light and dark shaded zones in Fig. 3. The beginning of settling was delayed at 4°C compared to room temperature. However, 90−95% of the suspension stayed completely turbid, and serious sedimentation was not observed for such a short period as 3 weeks even at room temperature, possibly resulting from no remarkable agglomeration. As a next step, the drying of nanosuspension was investigated in order to circumvent

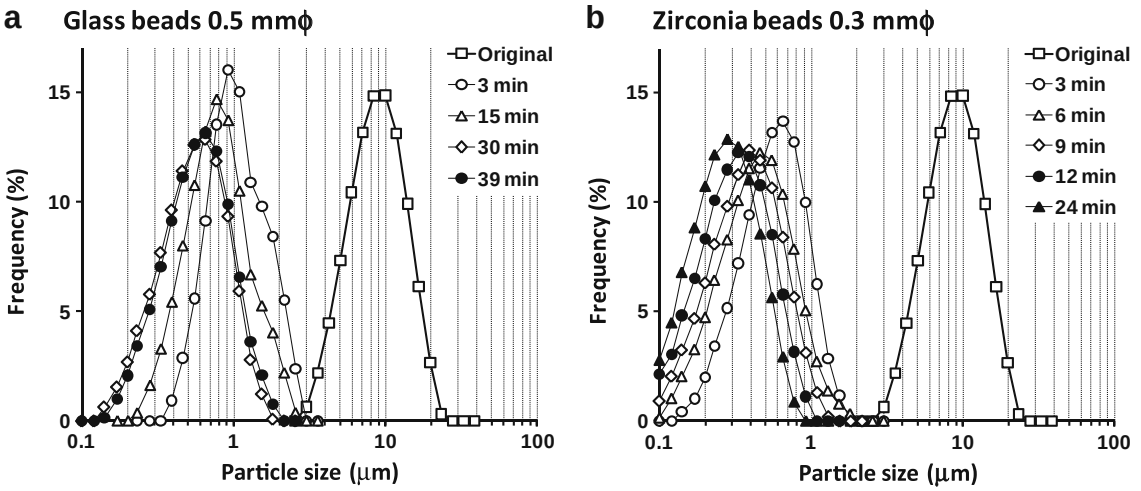

Fig. 2 Transition of particle size distribution during wet-milling process using (**a**) glass beads with 0.5 mmφ diameter and (**b**) zirconia beads with 0.3 mmφ diameter by oscillating beads method. 1200 mg of phenytoin was loaded in 15 mL of the dispersing medium and collected at preset time in the milling process.

these physical stability issues for longer periods and apply to the development of solid dosage forms.

Design of Dry Nanosuspension

The spray-drying method was applied to solidify the milled suspension in this research because this is one of the most popular drying processes in the pharmaceutical industry, and the solid components could be directly transformed to spherical particles. The resultant nanosuspension was supplied to the drying process in the following two approaches: 1) no other additive was formulated (Sus-SD), and 2) mannitol, as a nanoparticle carrier, was co-dissolved in spray suspension (Sus-SD+M). As a control, the solution composed of entirely the same formulation as Sus-SD, in which phenytoin was dissolved by adjusting pH to alkaline, was prepared and spray-dried (Sol-SD). The spray-drying products were successfully produced with high recovery (>70%) in all three formulations and with high reproducibility among batches. SEM photographs in Fig. 4 obviously demonstrate that the fine particles with single-micron diameter were obtained in all three cases (a-1, b-1, c-1). The spherical particles, which are recognized as a typical spray-dried product, were obtained from nanosuspension (a and a), whereas the irregular-shaped particles were formed from solution (c). The highly magnified photo (c-3) suggests

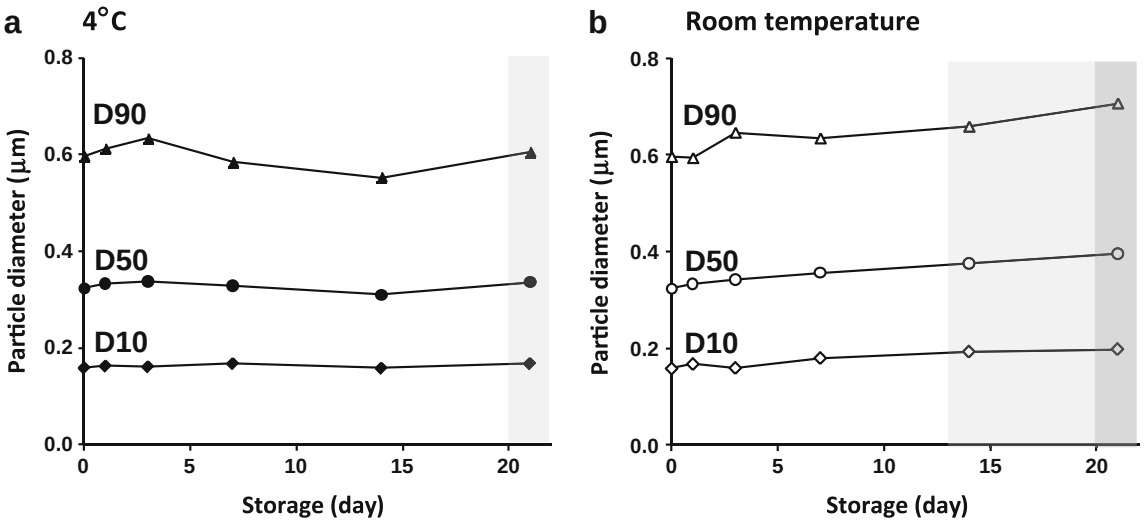

Fig. 3 Physical stability of nanosuspension from size distribution perspective during storage at (**a**) refrigerated and (**b**) ambient conditions. D10 (♦, ◊); D50 (●, ○); and D90 (▲, Δ) indicate the 10%, 50%, and 90% diameters on the cumulative volume distribution during storage at 4°C and room temperature, respectively. Clear zone indicates a nanosuspension that shows no settling, and light and dark shaded zones indicate a nanosuspension that shows a bit of settling, resulting in upper 5% and 10% parts of the total volume are slightly translucent, respectively.

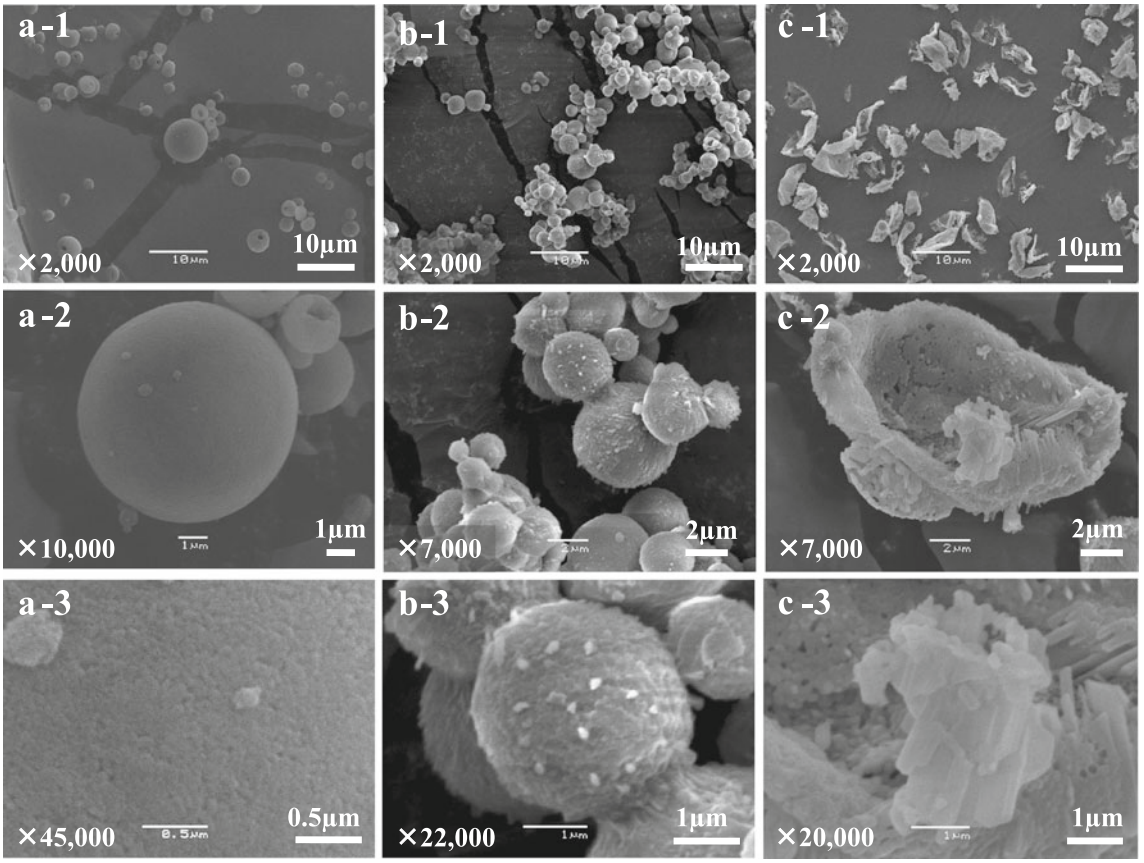

Fig. 4 Scanning electron microphotographs of spray-dried particles prepared from nanosuspension and solution of phenytoin. Appearance with 1) low, 2) middle, and 3) high magnification. (**a**) Sus-SD; (**b**) Sus-SD+M; (**c**) Sol-SD.

that phenytoin was finely crystallized and layered in the spray droplets, resulting in formation of particles with rugged structure. On the other hand, Sus-SD particle exhibited a perfect sphere with smooth surface macroscopically (a); in addition, the highly magnified view reveals the surface with mosaic structure assembled from lots of tiny pieces (a-3). Each piece was considered to be a milled particle of phenytoin because 87% weight in solidified mass was composed of phenytoin. In other words, each Sus-SD particle was considered to be a spherical aggregate composed of thousands of milled drug fragments. In the case of Sus-SD+M particles, many whisker-shaped protuberances were observed on the surface, assuming the crystallization of mannitol during drying process (b-3). The drug would be embedded in mannitol-based matrix. A part of particles seems to be cross-linked to next one, exhibiting a bunch of grapes as a whole. This solid bridge between particles would be attributed to the delayed crystallization of mannitol.

Physicochemical Characterization of Dry Nanosuspension

The particle size distributions of three spray-dried products shown in Fig. 5 were consistent with SEM observations. They were considerably overlapped and mainly located in the single-micron rage. The onset of distribution on the smaller side was 1.5 μm in all three samples, assuming to be related to the size of spray mists. The same driving conditions

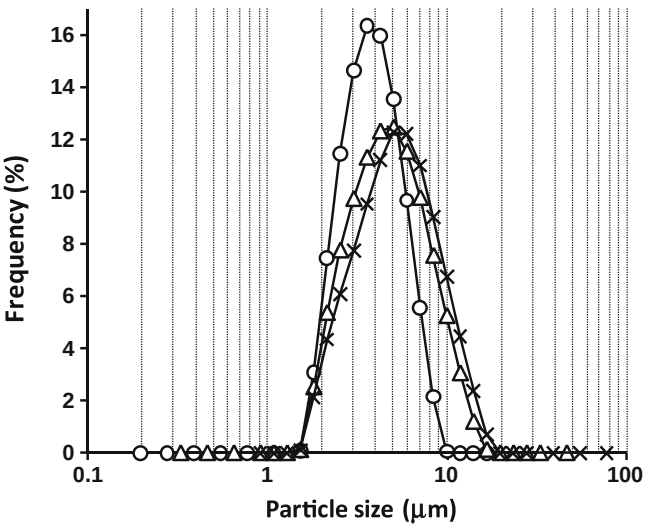

Fig. 5 Particle size distribution of each spray-dried particles. The spray-dried particles were dispersed in dry air under 0.4 MPa pressure. Key: (○) Sus-SD particles; (Δ) Sus-SD+M particles; (X) Sol-SD particles.

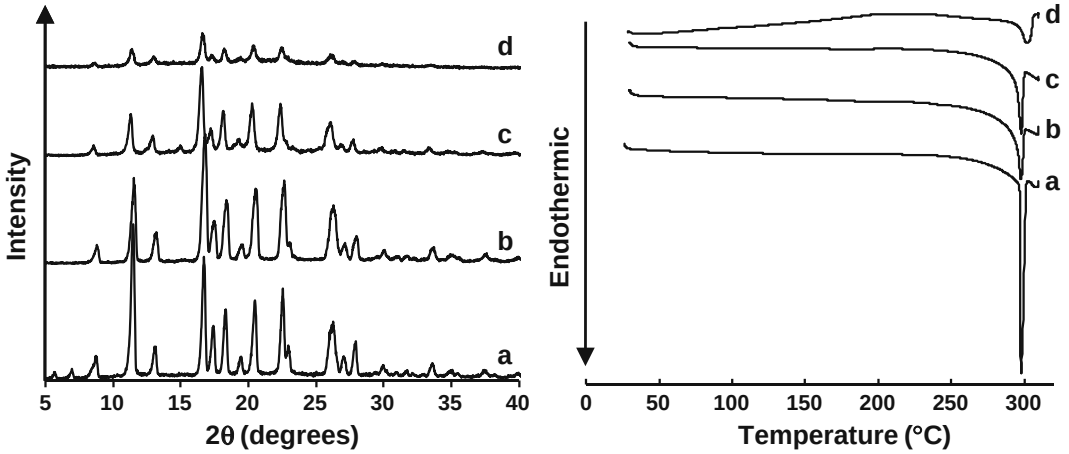

Fig. 6 X-ray powder diffraction patterns (*left*) and DSC profiles (*right*) of original bulk particles and each spray-dried particles from nanosuspension and solution of phenytoin. Key: (**a**) original bulk particles; (**b**) physical mixture of phenytoin and the additives with same composition as **c** and **d**; (**c**) Sus-SD; (**d**) Sol-SD.

of spray would probably form the equivalent size distribution of the mists. The Sus-SD particles were entirely distributed within single-micron range. However, the distribution curves of Sus-SD+M and Sol-SD particles were slightly widened to larger particle range, namely 15.5 and 17.1 μm of the largest end, respectively. As a result, the particles over 10 μm in size accounted for 0, 6.0, and 6.6% cumulatively in Sus-SD, Sus-SD+M and Sol-SD particles, respectively. As shown in Fig. 4b, some Sus-SD+M particles tended to be cross-linked, resulting in an increase the distribution. In case of Sol-SD particles, the cohesive behavior attributed to flat and irregular shape might promote the aggregation between particles during analysis. Conclusively, D50 values of Sus-SD, Sus-SD+M and Sol-SD particles were 3.47, 4.30 and 4.80 μm, respectively.

In order to investigate the effect of the loading state of phenytoin to be supplied to the spray-drying process on the crystalline property of the products, Sus-SD and Sol-SD particles, which have completely the same components and composition, were evaluated by X-ray powder diffraction (XRPD) and DCS. As shown in Fig. 6, the positions of diffraction peaks and the onset temperature at endothermic peak (297°C) of both particles were identical to those of the original phenytoin bulk, demonstrating that any polymorphic transformations might not occur during wet-milling and spray-drying processes. The reduced DCS endothermic peak in physical mixture as well as spray-dried samples suggested that a part of the drug would co-melt in liquefied additive below intrinsic melting point (297°C), assuming that it was difficult to evaluate the crystallinity quantitatively by DSC endothermic peak. The XRPD peak intensities of the Sus-SD particles were somewhat reduced from those of the physical mixture of the original materials (Fig. 6c), suggesting that the crystallinity of phenytoin was slightly weakened. However, this partial amorphization of the drug was more advanced in Sol-SD sample as detected by its much shorter diffractive peaks (Fig. 6d). Such diffractive results were not consistent with SEM observation in Fig. 4. That is, Sol-SD particle with shorter XRPD peaks appears to be a crystalline-like structure (Fig. 4); in contrast, Sus-SD particle with taller XRPD peaks looks amorphous in appearance (Fig. 4a). This inconsistency between X-ray crystallography and visual observation would be attributed to structural specificity of Sus-SD particle. As mentioned above, each particle was composed of thousands of crystalline nano-sized fragments. The spherical particle with smooth surface and

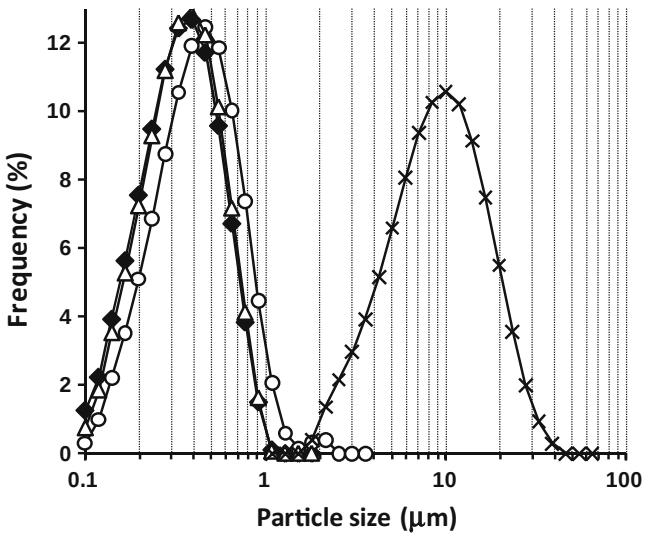

Fig. 7 Particle size distribution of the original nanosuspension and redispersed suspension of each spray-dried particles in water. The spray-dried particles were put into water and vortexed for 30 s to disperse to suspension. Key: (♦) original nanosuspension; (○) redispersed suspension from Sus-SD; (Δ) redispersed suspension from Sus-SD+M; (X) redispersed suspension from Sol-SD.

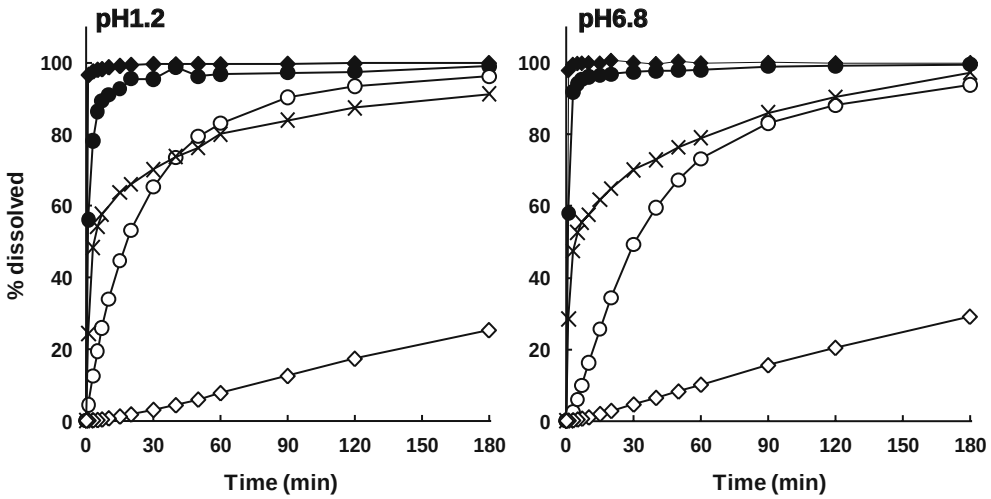

Fig. 8 Release profiles of phenytoin from spray-dried particles without mannitol and their physical mixture with same composition (left: pH1.2, right: pH6.8). Key: (◊) bulk particles of phenytoin; (♦) original nanosuspension; (●) Sus-SD particles; (X) Sol-SD particles; (○) physical mixture of Phe:PVP:SLS = 40:5:1.

high crystallinity would be simultaneously attained by micronization of drug crystals to nanometer size. Thus, it was found that the spray-dried products from nanosuspension are advantageous to maintain the crystalline state of the starting materials compared to those from solution. The crystalline peaks of phenytoin could also be confirmed in Sus-SD+M particles without changing their position (data not shown).

Pharmaceutical Performance of Dry Nanosuspension

Since the main attribute of the current technique is to increase dissolution rate resulting from the expanded surface area of the particles, the dispersion property of the products in aqueous medium is a key factor from a pharmaceutical perspective. The spray-dried products were soaked in water and vibrated for 30 s using voltex mixer. No ultrasonication was applied to promote the dispersion. The particle size distributions of redispersed suspension were shown together with original nanosuspension in Fig. 7. The spray-dried products from suspension (Sus-SD, Sus-SD+M) were well dispersed, and the homogeneous nanosuspension, whose distribution was almost the same as the original one, was reconstructed. It was remarkable that Sus-SD products could be entirely dispersed into nanoparticles with submicron range

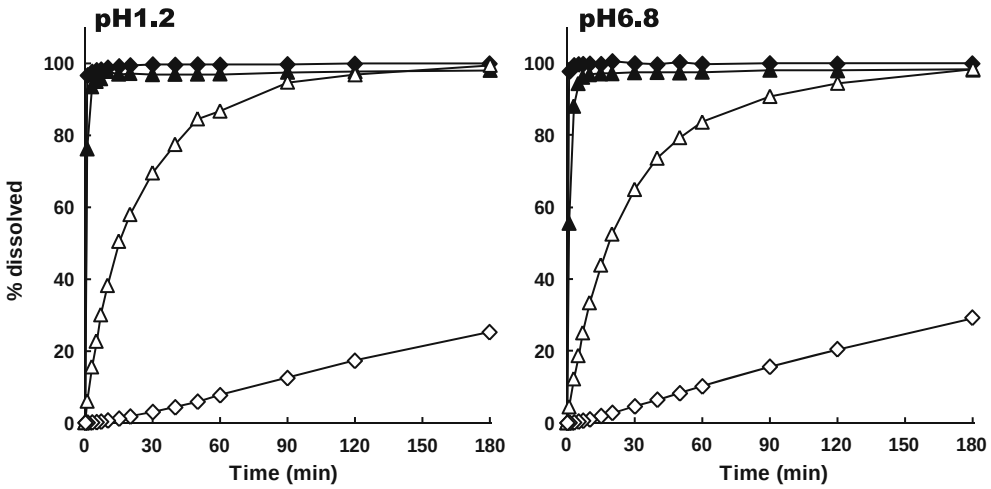

Fig. 9 Release profiles of phenytoin from spray-dried particles with mannitol and their physical mixture with same composition (left: pH1.2, right: pH6.8). Key: (◊) bulk particles of phenytoin; (♦) original nanosuspension; (▲) Sus-SD+M particles; (Δ) physical mixture of Phe:Man:PVP:SLS = 40:40:5:1.

although 87% weight in solidified mass was composed of phenytoin. The previous studies concluded that more than half the amount of additives, as a matrix former, should be formulated to protect the nanoparticle agglomeration effectively during drying (23,25). The present result suggested that additives were not always required if small amounts of effective dispersing agents (PVP and SLS) are coexisted. It was found that the high redispersiblility of Sus-SD products was reproducible among batches even if the conditions of spray-drying process were changed. It was also reported that the ternary ground mixture of drug/PVP/SLS could produce the colloidal drug nanoparticles when dispersing in water (34,35). From the human health point of view, it would be greatly advantageous that the nano-dispersion was achieved with much higher drug content (drug:PVP:SLS = 40:5:1) in this study compared to the previous one (drug:PVP:SLS = 1:3:1). The identical distribution between the original and redispersed Sus-SD+M suspensions suggested that mannitol was quite promotive to avoid the aggregate as a nanoparticle carrier. In contrast, the dried product from solution (Sol-SD) could not be further dispersed, but slightly aggregated in water, resulting in large and broad distribution ranging from 2 to 40 μm. This is the reason why the spray-dried powder, which has a potential to be spontaneously dispersible into primary milled particles and reconstructs the original nanosuspension, was named "dry nanosuspension" in this paper.

The dissolution tests of dried products were carried out in buffered media at pH 1.2 and 6.8 to simulate the gastrointestinal performance. The weighed samples were directly placed on the surface of the medium and rotated by a paddle. No specific operation was done to promote the dispersion. The release profiles of phenytoin from Sus-SD and Sus-SD+M particles were shown in Figs. 8 and 9, respectively. The fast onset of release was attained in both spray-dried products from the nanosuspension. In the both dissolution media, more than 95% of the drug was dissolved within 10 min after immediate homogeneous dispersion. The dispersion time for a few minutes or less was expressed as the slight delay from the dissolution of the original nanosuspension. The co-formulated mannitol accelerated the dispersion and following dissolution. With respect to Sol-SD product, the sustained release behaviors were observed in both media after rapid release up to around 50%. It was assumed that its low crystalline property would improve the dissolution at beginning stage, but its poor dispersibility shown in Fig. 7 would retard the subsequent dissolution. Consequently, the present technique using dry nanosuspension has great advantage that the dissolution of poorly water-soluble drug was significantly improved while maintaining high crystallinity.

CONCLUSION

In our previous study, the wet-milling technique in aqueous medium has been developed to prepare the oral nanosuspension focused on the discovery and preclinical animal studies for poorly water-soluble candidate compounds. In order to expand this technique into clinical and commercial applications, the spray-drying of resultant nanosuspension was investigated in this research. The spherical particles composed of thousands of nanometer-sized drug fragments were successfully prepared. The drugs in dried product were proved to be kept their original crystalline state, which is advantageous to provide the stable dosage from the physicochemical perspective. The spray-dried products from nanosuspension, named dry nanosuspension, could be spontaneously redispersed into the original nanosuspension and displayed immediate and perfect release behaviors in acidic and neutral media. Such excellent dispersion and release performances have not been achieved by the conventional spray-dried product form drug solution, demonstrating that the nanomilling in aqueous medium and the following spray-drying process would be a fundamental solubilization technique for poorly water-soluble drugs. In addition, the authors believe that the current technique could be widely applied throughout long pharmaceutical development, e.g. by utilizing as oral nanosuspension for animals in the discovery and preclinical researches, and as dry nanosuspension to clinical and commercial development stages. However, the potential risks for contamination derived from abrasion of beads should be addressed prior to clinical administration, which could be a common issue in such beads-milling technique. In addition, the robustness of high dispersiblility and release property should be also investigated on a larger manufacturing scale.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Isao Funahashi and Ms. Mai Mizutani for their excellent technical assistance throughout this work.

REFERENCES

1. Speiser PP. Poorly soluble drugs: a challenge in drug delivery. In: Müller RH, Benita S, Böhm B, editors. Emulsions and nanosuspensions for the formulation of poorly soluble drugs. Stuttgart: Medpharm Scientific; 1998. p. 15–28.
2. Prentis RA, Lis Y, Walker SR. Pharmaceutical innovation by seven UK-owned pharmaceutical companies. Br J Clin Pharmacol. 1988;25:387–96.
3. Lipinski CA. Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Meth. 2000;44:235–49.

4. Lipinski CA. Poor aqueous solubility − An industry wide problem in drug discovery. Am Pharm Rev. 2002;5:82–5.
5. Schroter C. Prioritizing molecules based on physicochemical characteristics. Am Pharm Rev. 2006;9:60–7.
6. Amidon GL, Lennernas H, Shah VP, Crison JRA. A theoretical basis for a biopharmaceutical drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res. 1995;12:413–20.
7. Friesen DT, Shanker R, Crew M, Smithey DT, Curatolo WJ, Nightingale JAS. Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview. Mol Pharmaceutics. 2008;5(6):1003–19.
8. Janssens S, Anné M, Rombaut P, Van den Mooter G. Spray drying from complex solvent systems broadens the applicability of Kollicoat IR as a carrier in the formulation of solid dispersions. Eur J Pharm Sci. 2009;35:241–8.
9. Ahmed IS, Aboul-Einien MH. *In vitro* and *in vivo* evaluation of a fast-disintegrating lyophilized dry emulsion tablet containing griseofulvin. Eur J Pharm Sci. 2007;32(1):58–68.
10. Gupta MK, Tseng YC, Goldman D, Bogner RH. Hydrogen bonding with adsorbent during storage governs drug dissolution from solid-dispersion granules. Pharm Res. 2002;19(11):1663–72.
11. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev. 2007;59:645–66.
12. Carrier RL, Miller LA, Ahmed I. The utility of cyclodextrins for enhancing oral bioavailability. J Controlled Release. 2007;123:78–99.
13. Kesisoglou F, Panmai S, Wu Y. Application of nanoparticles in oral delivery of immediate release formulations. Curr Nanosci. 2007;3:183–90.
14. Merisko-Liversidge EM, Liversidge GG. Drug nanoparticles: formulating poorly water soluble compounds. Toxicol Pathol. 2008;36:43–8.
15. Pouton CW. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and "self-microemulsifying" drug delivery systems. Eur J Pharm Sci. 2000;11:93–8.
16. Gursoy RN, Benita S. Self emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed Pharmacother. 2004;58:173–82.
17. Niwa T, Miura S, Danjo K. Universal wet-milling technique to prepare oral nanosuspension focused on discovery and preclinical animal studies—Development of particle design method. Int J Pharm. 2011;405:218–27.
18. Parikh D. Spray drying as a granulation technique. In: Parikh D, editor. Handbook of pharmaceutical granulation technology, drugs and the pharmaceutical sciences. New York: Marcel Dekker; 1997. p. 75–96.
19. Cal K, Sollohub K. Spray drying technique. I: Hardware and process parameters. J Pharm Sci. 2010;99(2):575–86.
20. Sollohub K, Cal K. Spray drying technique: II. Current applications in pharmaceutical technology. J Pharm Sci. 2010;99(2):587–97.
21. Merisko-Liversidge E, Sarpotdar P, Bruno J, Hajj S, Wei L, Peltier N, *et al.* Formulation and antitumor activity evaluation of nanocrystalline suspensions of poorly soluble anticancer drugs. Pharm Res. 1996;13(2):272–8.
22. Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: A formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci. 2003;18:113–20.
23. Van Eerdenbrugh B, Froyen L, Martens JA, Blaton N, Augustijns P, Brewster M, *et al.* Characterization of physico-chemical properties and pharmaceutical performance of sucrose co-freeze-dried solid nanoparticulate powders of the anti-HIV agent loviride prepared by media milling. Int J Pharm. 2007;338:198–206.
24. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, Martens JA, Augustijns P, Van den Mooter G. Drying of crystalline drug nanosuspensions − The importance of surface hydrophobicity on dissolution behavior upon redispersion. Eur J Pharm Sci. 2008;35:127–35.
25. Van Eerdenbrugh B, Froyen L, Van Humbeeck J, Martens JA, Augustijns P, Van Den Mooter G. Alternative matrix formers for nanosuspension solidification: dissolution performance and X-ray microanalysis as an evaluation tool for powder dispersion. Eur J Pharm Sci. 2008;35:344–53.
26. Jacobs C, Müller H. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm Res. 2002;19(2):189–94.
27. Hecq J, Deleers M, Fanara D, Vranckx H, Boulanger P, Le Lamer S, *et al.* Preparation and *in vitro/in vivo* evaluation of nano-sized crystals for dissolution rate enhancement of ucb-35440-3, a highly dosed poorly water-soluble weak base. Eur J Pharm Biopharm. 2006;64:360–8.
28. Junghanns JAH, Müller RH. Nanocrystal technology, drug delivery and clinical applications. Inter J Nanomedicine. 2008;3 (3):295–309.
29. Tanaka Y, Inkyo M, Yumoto R, Nagai J, Takano M, Nagata S. Nanoparticulation of poorly water soluble drugs using a wet-mill process and physicochemical properties of the nanopowders. Chem Pharm Bull. 2009;57(10):1050–7.
30. Kawabata Y, Yamamoto K, Debari K, Onoue S, Yamada S. Novel crystalline solid dispersion of tranilast with high photostability and improved oral bioavailability. Eur J Pharm Sci. 2010;39(4):256–62.
31. Gulsun T, Gursoy RN, Oner L. Design and characterization of nanocrystal formulations containing ezetimibe. Chem Pharm Bull. 2011;59(1):41–5.
32. Tewa-Tagne P, Briançon S, Fessi H. Preparation of redispersible dry nanocapsules by means of spray-drying: Development and characterisation. Eur J Pharm Sci. 2007;30:124–35.
33. Grant DJW, Brittain HG. Solubility of pharmaceutical solids. In: Brittain HG, editor. Physical characterization of pharmaceutical solids, drugs and the pharmaceutical sciences, vol. 70. New York: Marcel Dekker; 1995. p. 321–86.
34. Pongpeerapat A, Wanawongthai C, Tozuka Y, Moribe K, Yamamoto K. Formulation mechanism of colloidal nanoparticles obtained from probucol/PVP/SDS ternary ground mixture. Int J Pharm. 2008;352:309–16.
35. Moribe K, Wanawongthai C, Shudo J, Higashi K, Yamamoto K. Morphology and surface states of colloidal probucol nanoparticles evaluated by atomic force microscopy. Chem Pharm Bull. 2008;56(6):878–80.