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Preparation and Characterization of an Oridonin Nanosuspension for Solubility and Dissolution Velocity Enhancement

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This study describes the preparation of an oridonin (ORI) nanosuspension by high-pressure homogenization (HPH). The aim was to obtain a stable nanosuspension with an increased drug saturation solubility and dissolution velocity. The homogenization procedure was optimized in regard to particle size and long-term stability. The characteristics of the oridonin nanosuspension, such as particle size, size distribution, shape, and zeta potential, were evaluated following the water removal. The solubility and dissolution experiments were performed to verify the obvious improvement of the dissolution behavior compared with commercial ORI. Finally, crystalline state evaluation before and following the formulation was performed through differential scanning calorimetry (DSC) and powder X-ray (PXRD).

Keywords nanosuspensions; poorly soluble drugs; high-pressure homogenization; oridonin

INTRODUCTION

It is well known that many drugs directly synthesized or extracted from natural plants are poorly soluble in water, consequently in biological media. Therefore, the majority of these drugs have a poor bioavailability after oral administration. Parenteral routes (e.g., intraperitoneal, intramuscular, etc.) through the conventional approaches are also of limited success for these drugs, because of either the dissolution problem of microsuspensions in the local tissue fluid or the adverse effect/toxicity caused by the solvent mixture or co-solvents used in the injection. Recently, a carrier-free colloidal drug delivery system—nanocrystal suspensions—has been applied to tackle the formulation issue of poorly soluble drugs. Nanocrystal suspension, called nanosuspension for short, is a carrier-free nanoparticle system containing only pure drug crystals and minimum stabilizers for stabilization (Keck & Muller, 2006). At present, several production techniques have been applied to produce drug nanosuspensions, such as precipitation (Siostrom, Kronberg, & Carlfors, 1993), pearl milling (Liversidge, Liversidge, & Cooper, 2003), and high-pressure homogenization (HPH) (Hecq, Deleers, Fanara, Vranckx, & Amighi, 2005; Krause & Muller, 2001). Among these techniques, the HPH method, pioneered by Muller in the 1990s, has shown great superiority over other methods (Muller, Jacobs, & Kayser, 2001). The outstanding feature of nanosuspensions is significantly increased drug solubility and dissolution velocity due to the small particle size and enormous particle surface following the nanosizing process. In addition, the toxicity and adverse effects caused by some solubilizers could be avoided by using the nanosuspension system. As a versatile delivery system, nanocrystal suspensions may be formulated for rapid dissolution following pharmacokinetic properties similar to those of a solution (Clement, Pugh, & Parikh, 1992), or drug insolubility may be adjusted to afford prolonged in vivo release (White et al., 2003); it also can target infection sites or organs following phagocytosis by the phagocytic cells of the reticuloendothelial system (RES) of the circulatory system (Peters et al., 2000). At present the nanosuspensions are

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mainly used to increase the saturation solubility and dissolution velocity of the poorly soluble drugs, with the purpose of elevating their bioavailability and efficiency.

Oridonin (ORI) (Figure 1) is an active diterpenoid compound extracted from the Chinese traditional medicine *Rabdosia rubescens* (Osawa et al., 1994; Zhang, Han, Zhao, & Sun, 2003). Pharmacological experiments show that ORI has obvious anti-cancer activities (Zhang & Ren, 2003), and clinical data suggest that it effectively inhibits the proliferation of a wide variety of cancer cells, including those from the liver, prostate, breast, and cervix; non–small cell lung cancer; acute promyelocytic leukemia; and glioblastoma multiforme (Chen et al., 2005; Ikezoe et al., 2003; Zhang et al., 1999). It has been successfully used for treatment against liver cancer and esophageal carcinoma in the clinical setting for decades (Fujita et al., 1988; Zhang & Ren, 2003).

However, its preparation has not exhibited perfect clinical effects due to its poor solubility and low bioavailability. The traditional approach to tackling this issue is the use of solubilizing agents such as ethanol and Tween 80 to obtain complete solution of ORI (Liu & Zhao, 1998), but adverse effects such as inflammation of the blood vessel and topical pain caused by the miscible agents limited its use in the clinic (Zhang & Ren, 2003). A 10.5-fold increase in solubility could be achieved by the production of ORI-cyclodextrin complex (Zhang, Kou, Lu, & Wang, 2001), but large quantities of cyclodextrin might be required due to the high molecular weight of cyclodextrin and excess quantities of cyclodextrin added to drive the equilibrium toward complexation; the high levels of excipients make administration inconvenient, especially oral application (Kipp, 2004). In recent decades, submicron carrier delivery systems, such as polymer nanoparticles (Xing, Zhang, & Tan, 2007) and solid lipid nanoparticles (Zhang, Tan & Gao, 2007), were applied to overcome the problem in our research. Although fine-targeting behavior and in vivo pharmacokinetic characteristics were obtained through these carrier systems, the remnant organic solvents in the final products and considerable surfactants used in the progress were adverse to the body. In addition, the procedure for preparing carrier nanoparticles was very complicated, time consuming, and not readily scaled up. Hence there is a growing need for a versatile formulation approach that can tackle the formulation-related problems associated with the ORI.

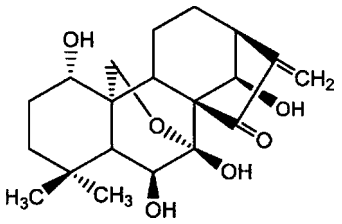

FIGURE 1. Chemical structure of ORI.

In this study, the hydrophobic drug ORI was formulated as a nanosuspension by HPH method to overcome the formulation problem due to its poor dissolution behavior. The effects of kinds and amount of stabilizers on stabilities of nanosuspension were investigated and the optimum stabilizers for ORI nanosuspensions were obtained through the screening of formulation in regard to particle size and long-term stability. The physicochemical properties of the ORI nanosuspensions were evaluated, including the particle size, size distribution, and zeta potential. Much attention was given to investigating dissolution kinetics of ORI from nanocrystal powder. ORI crystalline state was also evaluated before and following particle size reduction to confirm that the initial crystalline state was maintained. This method was simple, cost effective, time saving and easily scaled up, and could be applied to most poorly soluble drugs.

MATERIALS AND METHODS

Materials

ORI (99%) was purchased from Shanxi Huike Plants Exploitation Co., Ltd; pluronic F_{68} from Brij 78, PVP K25; sodium dodecyl sulphate (SDS) from Sigma (USA); lecithin from Beijing Shuangxuan Microbic Medium Co., Ltd; and mannitol, sodium hydroxide, and potassium dihydrogen phosphate from a Shanghai chemical agent company water used in the experiment was deionized, and other materials were of analytical reagent.

Preparation of ORI Nanosuspensions

The stabilizers were dissolved or dispersed in bi-distilled water using an Heidolph homogenizer (DIAX 900, Germany) until the stabilizers had completely dissolved or were finely dispersed. Then the ORI powder was poured into the stabilizer solution (1% ORI, w/v); the resultant suspensions were sufficiently dispersed using the Heidolph homogenizer at 10,000 rpm for 1 minute. Then the nanosuspensions were prepared using a piston-gap homogenizer (NS1001L, Italy). First, premilling steps were conducted on the suspensions to decrease the particle size at 200 bar and 500 bar, respectively, for 2 cycles. Then an HPH step was applied on the suspensions at 1500 bar for 20 cycles. At this step samples after every cycle were withdrawn for particle size analysis.

Water Removal

To increase the shelf life of the nanosuspensions and to further study the dissolution behavior and physical state of the formulation, the suspensions were freeze-dried immediately after preparation. First the suspensions were poured into glass flasks and pre-frozen using an ultra-cold freezer (MDF-382E, SANYO, Japan) at –80° for 24 hours; then the samples were freeze-dried using a lyophilizer (LGJ 0.5, Beijing, China) at –40° and at 0.10 mbar of pressure for 48 hours to yield dry powder.

Mannitol (1%, w/v) was added into the suspensions prior to freezing as cryoprotectant.

Particle Size Distribution and Morphological Characterization

The size distribution of the samples was measured using Zetasizer (3000SH, Malvern Instruments Ltd., UK) after dilution with the ORI-saturated water. The morphology of the nanocrystals was observed with an XSP-2C light microscope (YS2-H, Nikon, China) and a scanning electron microscope (ETEC-Autoscan, Siemens, Germany).

Zeta Potential Measurement

The electrophoretic mobility and the zeta potential of the nanosuspensions were analyzed by the Zetasizer after dilution with the ORI-saturated water.

Solubility

To confirm the increase in the solubility of the ORI nanocrystal powder compared with the commercial ORI, saturation solubility was determined using a constant-temperature shaker (THX-82, Shanghai, China). Samples containing equivalent ORI (20 mg) were dispersed into 50 mL phosphate buffered solution (PBS, pH = 7.4) used as the solvent to simulate the in vivo environment, and the temperature and shake rate were set to 37° and 100 min^{-1}, respectively. Three mL of medium was withdrawn at predetermined intervals and filtered using an ORI-saturated 0.22-μm micropore film (Q/IEFJ01-1997, Shanghai, China). After properly diluted, the samples were assayed through ultraviolet absorbance determination at 238.0 nm using an ultraviolet detector (UV-2102, Unico, USA). Each sample was analyzed in triplicate.

Dissolution

Dissolution behavior of the ORI nanocrystal powder in vitro was estimated using a dissolution apparatus (RC-3B, Tianjin, China) with the paddle method. Samples containing equivalent of ORI (10 mg) were dispersed into 900 mL PBS (7.4) (containing 5% ethanol), which was used as the release medium to simulate the in vivo environment; the temperature and rate were set to 37° and 100 min^{-1}, respectively. At predetermined intervals, 5 mL of medium was withdrawn, then filtered with an ORI-saturated 0.22-μm micropore film, and equal blank medium was compensated immediately to sustain the sink condition. The amount of dissolved drug was determined by ultraviolet analysis at 238.0 nm. Each sample was analyzed in triplicate.

Assessment of Crystalline State

Differential Scanning Calorimetry (DSC)

Thermal properties of the powder samples were investigated with a differential scanning calorimeter (CDR-4P, Shanghai, China). Approximately 10 mg of sample was analyzed in open aluminum pans and heated at scanning rate of 10° C/minute between 25° C and 600° C. Magnesia was used as the standard reference material to calibrate the temperature and energy scale of the DSC apparatus. To evaluate the internal structure modifications of the drugs before and after the formulation, thermal analysis was performed on ORI, pluronic F_{68}, lecithin, their physical mixtures, and the freeze-dried ORI nanosuspension powder.

Powder X-Ray (PXRD)

The crystalline state of the samples was estimated by a X-ray diffractometer (D/max r-B, Rigaku, Japan). Diffraction of each of the excipients, their physical mixtures, and ORI dried nanosuspension powder was researched with a Cu line as the source of radiation. Standard runs using a 40 kV voltage, a 40 mA current, and a scanning rate of 0.02° min^{-1} over a 2θ range of 2.5° to 40° were used.

RESULTS AND DISCUSSIONS

HPH is a very simple progress; through it we obtained a homogenous and stable nanosuspension system of ORI using a piston-gap homogenizer. In the piston-gap homogenizer, the raw material suspensions (in micro size) are pumped from the pipe into a gap (25 μm at 1500 bar). The high energy provided by the pump converts to increased kinetic energy as the suspensions pass through the narrow gap. According to the Bernoulli's equation, the static pressure on the fluid simultaneously decreases when the fluid pass through the gap at a high speed. The water starts boiling as the static pressure falls below the boiling of water, gas bubbles are formed which implode vigorously when the suspensions leaves the gap, and cleavage along the crystal dislocation occur, caused by cavitation, fluid shear, and particles colliding against each other. The energy due to cavitational bubble collapse can be enough to break down the drug microparticles into nanosized particles (Muller, Becker, Kruss, & Peters, 1999).

Screening of the Parameters of Production

Figure 2 shows that in the previous experiment, smaller and smaller particle sizes of ORI were obtained with increased pressure and number of cycles. Therefore, for a specified drug the particle size is a function of production parameters including pressure and cycles. A higher pressure means that the energy conducted on drug particles is higher, so the crystalline defect that cannot be cracked at lower pressure can be broken down. The increased cycles also provided more energy to break down the crystals. For each drug, a minimum size exists that can be achieved at a certain pressure with the increased cycles. When the minimum value is achieved, more cycles cannot lead to a continually reduced particle size unless higher pressure is applied (Muller et al., 2001). Usually, because of

the narrowness of the homogenizer gap, a procedure called premilling should be conducted on the suspensions to prevent blockage of the gap before the homogenization; that is, the application of the low pressure at the beginning. The homogenization gap is larger at lower pressure, thus allowing even larger crystals to be processed (Moschwitzera, Achleitnerb, Pomperb, & Muller, 2004). Considering the procedure property and pre-experiments, in our optimized procedure we prepared the ORI nanosuspensions first using the pressure of 200 bar and then 500 bar for 2 cycles, respectively, then the high pressure of 1,500 bar was applied to the suspensions for 20 cycles. Besides simplicity, it also could be concluded from Figure 2 that the HPH method had a good reproducibility because of a small standard deviation.

Screening of the Formulation

In the preliminary experiments we used a different type of stabilizer with a fixed stabilizer-to-drug ratio (1:5) to select an optimal stabilizer for ORI. The mean particle size of the ORI nanocrystals stabilized with different stabilizer at 4° over a long period of time is shown in Figure 3 (no preservatives were added). It was indicated that for ORI, the mixture of pluronic F_{68} and lecithin (3:1) had the best effect on the long-term stability of the system. The amount of the stabilizer was also

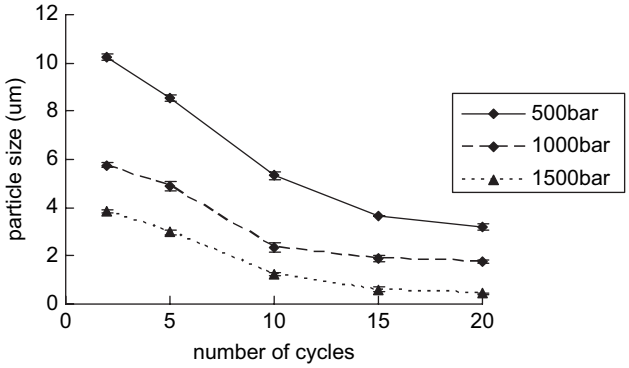

FIGURE 2. Influence of different applied pressures and cycle number on the particle size of the ORI nanosuspensions (1% ORI dispersed in a stabilizer solution containing 0.15% pluronic F_{68} and 0.05% lecithin) (n = 5).

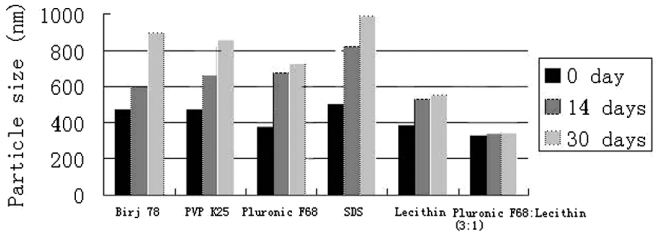

FIGURE 3. Influence of different stabilizers on particle size of ORI nanocrystals during a long shelf time at 4°.

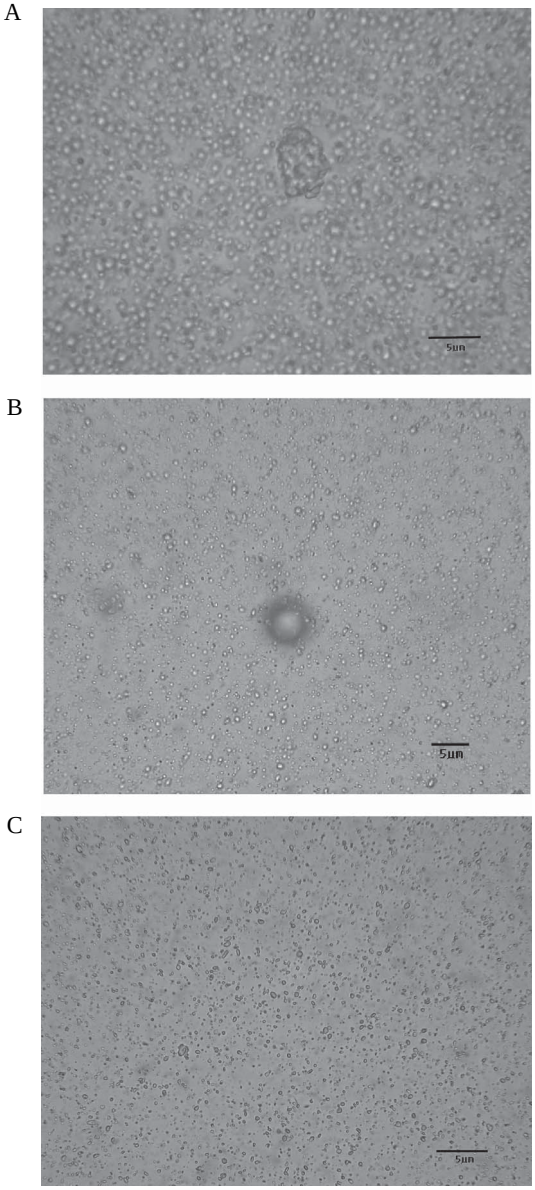

FIGURE 4. Light microphotos (100×10, scale = 5 μm) of the ORI nanosuspensions prepared in different stabilizer-to-drug ratios—A: 1:10; B: 1:3; C: 1:5.

investigated in our preliminary experiments. Figure 4 shows light microphotographs of formulations with different stabilizer-to-drug ratios (see Table 1). Adherence between the particles, caused by insufficient coverage of the stabilizer on the surface, can be viewed in Figure 4a. However, when the stabilizer-to-drug ratio rises to 1:3, micelles were formed by the excess stabilizers; this can be viewed in Figure 4b. Figure 4c shows that when the ratio was leveraged to a proper value (1:5), the nanocrystals viewed under a light microscope were dispersed with a uniform size and without any adherence and aggregation. Hence formulation C was the optimized one and was used for the next evaluation of the characteristics.

TABLE 1
Composition of ORI Nanosuspensions

Formulation	Ori (w/v)	Stabilizer-to-Drug Ratio	Mannitol (w/v)	Deionized Water (mL)
A	1%	1:10	1%	100
B	1%	1:3	1%	100
C	1%	1:5	1%	100

Stabilizers were added into the suspensions to prevent the aggregation or agglomeration of the nanocrystals. The physical instability of the system arises from the natural tendency of the nanoparticles to reduce the high surface energy created by the large interface between the solid and the medium. The choice of stabilizer is specific to each drug, and the type and amount of stabilizer also have a great effect on the physical stability of the nanosuspensions (Kocbek, Baumgartner, & Kristl, 2006). Firstly, the stabilizer should have a sufficient affinity for the particle surface of the special drug in order to stabilize the nanosuspensions (Lee, Lee, Choi, Yoo, & Ahn, 2005). Secondly, the stabilizers absorbed by the particle surface could provide sufficient repulsion between particles against aggregation. ORI is a nonpolar molecule, so ionic stabilizers such as sodium dodecyl sulphate (SDS) could not provide sufficient affinity with the particle surface, and ideal stability could not be obtained by using this kind of stabilizer alone. On the contrary, the non-ionic stabilizers, such as pluronic F_{68}, Birj 78, and PVP, may show a high affinity by possessing multiple attachment of hydrophobic domains at the surface, which could adequately interact with the hydrophobic functional groups of ORI (Kipp, 2004), but the steric barrier provided by these kinds of stabilizers might not be enough to prevent the aggregation of particles. Lecithin showed a better effect on stabilization of nanosuspension because the high affinity of its hydrophobic functional and the high electrostatic barrier provided by its ionic functional group. An optimum stabilization could be obtained by using a mixture of different kinds of stabilizers in this study (shown in Figure 3). Similar results were reported by Muller and Jacobs (2002). The amount of stabilizer should be sufficient for full coverage of the particle surface to provide enough electronic or steric repulsion between the particles. It not true that the more stabilizer the better; excess stabilizer tends to form micelles containing a small number of dissolved drug molecules (shown in Figure 4). Similar results were reported by Choi, Yoo, Kwak, Nam, and Lee (2005).

Before water removal, 1% mannitol, used as cryoprotectant, was added to the formulation. It recrystallized around the nanocrystals during the water removal progress to prevent particle agglomeration. This highly water-soluble compound also created a highly hydrophilic environment around the ORI nanocrystals, which was beneficial to increasing the dissolution of the drugs.

Morphology and Particle Distribution

The nanocrystals had a slice-like shape under the electron microscope, as shown in Figure 5. Figure 6 shows a narrow size distribution of the ORI nanocrystals in the suspension with a mean particle size of about 322.7 nm. Aggregation and overlap of the crystal slices could be found in the SEM micrographs, but the nanocrystal powder could be easily formulated to an uniform submicron system following redispersation in the water, which could be verified by the narrow size distribution in

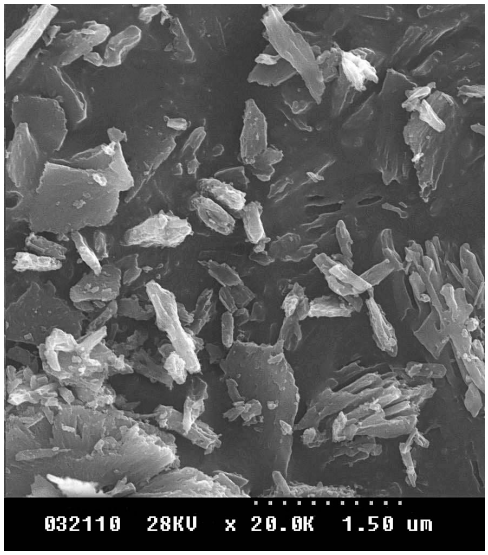

FIGURE 5. SEM micrographs of ORI nanocrystals (× 20,000, scale = 1.50μm).

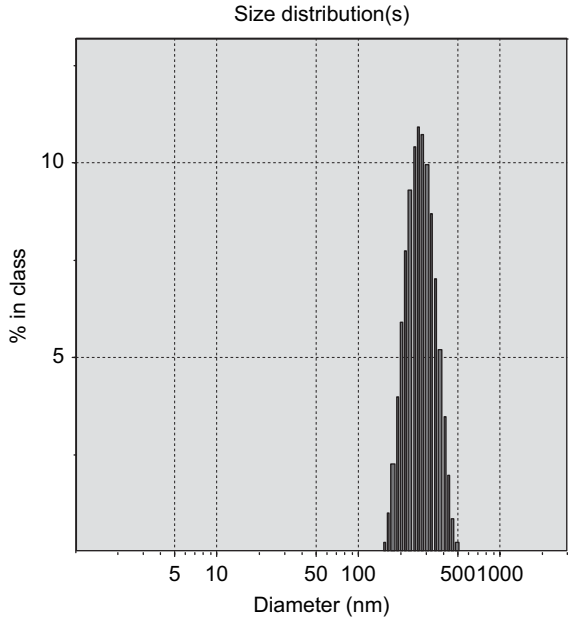

FIGURE 6. Size distribution of the ORI nanosuspensions in formulation C.

Figure 6. Uniform size or narrow size distribution was very important for the long-term stability of the nanosuspensions. It was reported that the particles in the dispersed systems tended to grow, a result of the differences in saturation solubility between small and large particles. The phenomenon is called Ostwald ripening (Jacobs, Kayser, & Muller, 2000). It could be explained by Ostwald-Freundlich's equation Equ (1):

$$\ln \frac{S}{S_0} = \frac{2M\gamma}{\rho r R T} \quad (1)$$

S is the saturation solubility, S_0 is the solubility of a flat sheet ($r = \infty$), M is the molecular weight of the solid, γ is the interfacial tension, R is the gas constant, T is the absolute temperature, r is the radius, and ρ is the density of the solid.

It is evident from Equ (1) that besides the temperature, the solubility of the substance is also a function of the particle size, larger particles having a higher solubility. However, this effect is only pronounced for particles below 1 μm (Liversidge et al., 2003). Therefore, in a particle-dispersed system, the solute concentration is higher in the vicinity of the smaller particles than that of the larger ones due to the higher saturation solubility of the small particles. So the solutes will diffuse from the surface of the small particles to the surrounding of the large particles driven by the concentration gradient, and recrystallize on the surface of the larger particles. The continual dissolution of the small particles and recrystallization of the solute on the surface of the large particles led to the formulation of the microparticles. However, the narrow size distribution conduced to the absence of the Ostwald ripening by eliminating the different solute concentration among the medium. So the nonuniformity of the particle size is another factor influencing the stability of the nanosuspensions besides the high surface energy.

Zeta Potential

Measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion (Li, Dong, Jia, Chang, & Xue, 2006). In general, particle aggregation is less likely to occur if particles possess enough zeta potential to provide sufficient electric repulsion or enough steric barrier to provide sufficient steric repulsion between each other. According to the literature, a zeta potential of at least –30 mV for electrostatically and –20 mV for sterically stabilized systems is desired to obtain a physically stable nanosuspension (Jacobs et al., 2000). The zeta potential of formulation C was -26.74 ± 2.68 mV ($n = 9$). So formulation C, expected to be a stable preparation, was coincident with the results shown in Figure 3.

Dissolution and Solubility Behavior

Figure 7 demonstrates that the ORI nanocrystals, when compared with the commercial ORI powder, noticeably

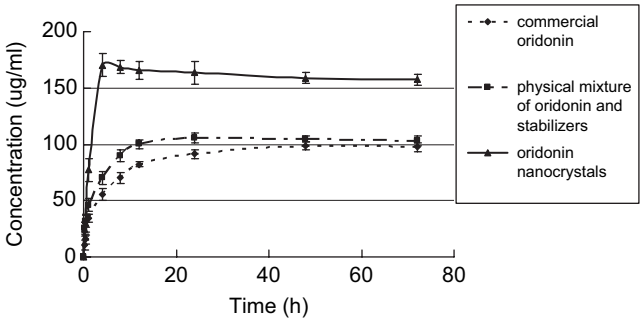

FIGURE 7. Profiles of saturation solubility achievement at 37° for commercial ORI, physical mixture of ORI and stabilizers, and ORI nanocrystal powder ($n = 3$).

increased saturation solubility of the drug. The commercial ORI showed a solubility of 99 ± 2 μg/mL at about 48 hours. For the ORI nanocrystals, a concentration of 170 ± 10 μg/mL could be achieved after 4 hours, which decreased slowly with time, indicating achievement of super-saturated ORI in the medium. The surfactants pluronic F_{68} and lecithin could accelerate the dissolution of the ORI, however, their solubilization effect only led to a small increase in solubility. So the obvious increase of the drug was attributed to the formulation of the nanosizing crystals. This result was consistent with Equ (1).

Besides the increase in saturation solubility, enhancement of the dissolution rate was also achieved for the nanocrystals. Figure 8 shows the dissolution profiles of the commercial ORI and samples after 5, 10, and 20 cycles at 1,500 bar. This indicated that dissolution velocity of ORI increased with the decreased particle radius. The percents of accumulated dissolved commercial particles, the particles after 5 cycles, and 10 cycles at 2 hours were 40.3%, 44.2%, and 66.5%, respectively. However, the particles in the nanocrystals dissolved 98% of the drugs at 24 minutes. From the Nernst-Brunner and Levich equation (Equ [2], a modification of the Noyes-Whitney equation), we knew that the increased surface area (A) and

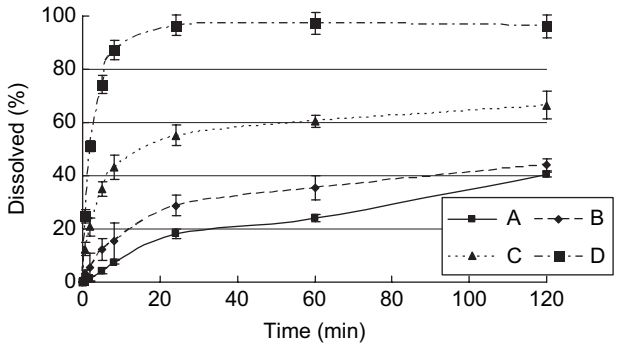

FIGURE 8. Dissolution profiles for commercial ORI (A) and HPH products after 5 cycles (B), 10 cycles (C), and 20 cycles (D).

saturation solubility (C_S) due to the decreased radius led to the increased dissolution velocity:

$$\frac{dX}{dt} = \frac{DA}{h} \times (C_S - \frac{X}{V}) \quad (2)$$

dX/dt is the dissolution rate, D is the diffusion coefficient, A is the surface area of the particle, h is the diffusional distance, C_S is the saturation solubility of the drug, X is the concentration in the surrounding liquid, and V is the volume of the dissolution medium.

In conclusion, formulation of poorly water-soluble drugs as nanometer-sized drug particles has a dramatic effect on dissolution rate, drug solubility, and consequently, bioavailability. A classic example is danazol, a poorly soluble gonadotropin inhibitor (Liversidge & Cundy, 1995). The absolute bioavailability of the marketed danazol microsuspension Danocrine was only 5.2%. When administered as a nanosuspension, an absolute bioavailability of 82.3% could be achieved.

Crystalline State

The assessment of the crystalline state helps in understanding the polymorphic changes that drugs might undergo when subjected to nanosizing. So it is essential to investigate the extent of amorphous state generated during the production of nanosuspensions (Patravale, Date, & Kulkarni, 2004). Figure 9 exhibits the DSC thermograms of ORI, pluronic F_{68}, lecithin, their physical mixtures, and ORI nanocrystal powder. Characteristic peaks of ORI (491.0º, A), pluronic F_{68} (91.2º, 305.0º, B), could be found in ORI nanocrystal (E) as well as in the physical mixture profile (D), meaning the drug and its excipients remained in their initial crystalline state. The PXRD analysis (Figure 10) shows the similar condition; that is, the characteristic peaks of ORI (at 2θ of 15.2º and 23.1º) and pluronic F_{68} (at 2θ of 18.93º and 23.2º) could be found in the profile of ORI nanocrystal powder. This result confirmed that the HPH progress had no influence on the ORI crystalline state, and the enhancement of dissolution rate of ORI was due to the reduction of particle size but not the appearance of amorphous form.

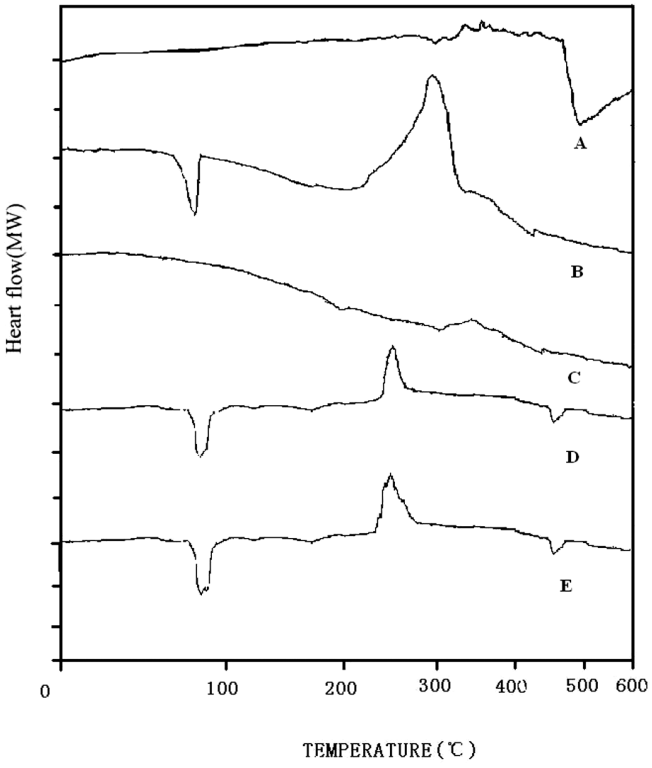

FIGURE 9. Differential scanning calorimetry thermograms of ORI (A); Pluronic F_{68} (B); lecithin (C); physical mixture of ORI, Pluronic F_{68}, and lecithin(D); and ORI nanocrystal powder (E).

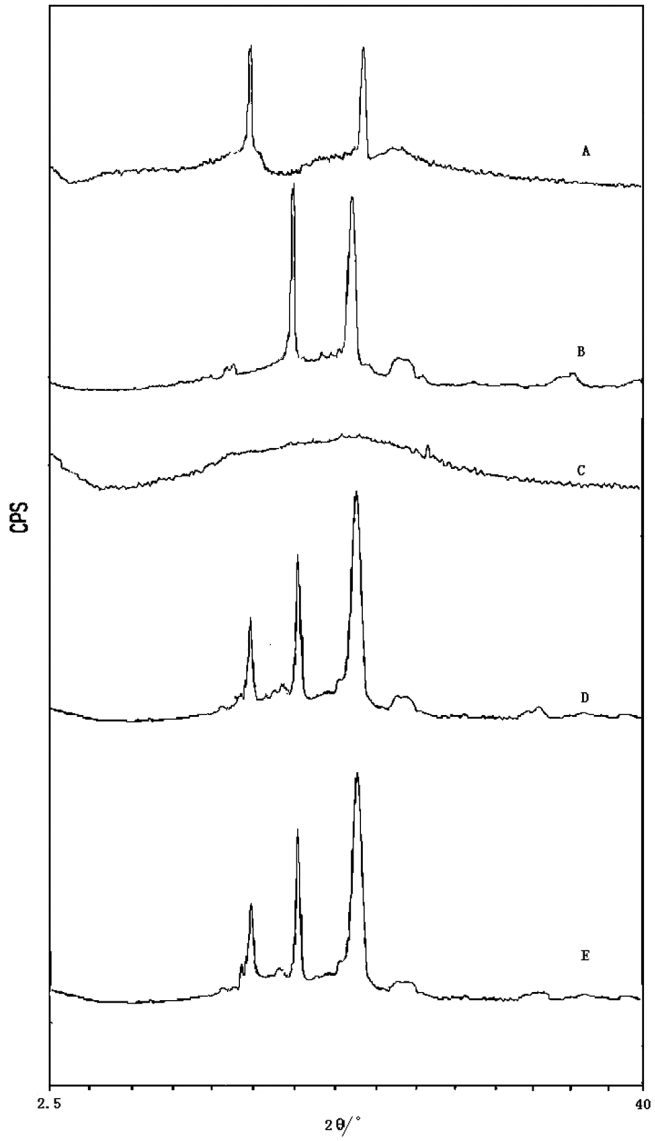

FIGURE 10. X-ray scattering of ORI (A); Pluronic F_{68} (B); lecithin (C); physical mixture of ORI, pluronic F_{68}, and lecithin (D); and ORI nanocrystal powder (E).

Furthermore, maintenance of initial crystalline state was profitable for a long-term stability.

CONCLUSIONS

In this study, it has been shown that formulation of ORI as a nanocrystal suspension has exhibited great success in dissolution rate and saturation solubility enhancement due to its size and enormous surface area. The HPH method was shown to be a simple and efficient technique for particle size reduction with the help of optimized stabilizers. Furthermore, the crystalline state of ORI was not altered through the process of particle size reduction, which is beneficial for the long-term stability of ORI nanosuspensions.

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REFERENCES

Chen, S., Gao, J., Halicka, H. D., Huang, X., Traganos, F., & Darzynkiewicz, Z. (2005). The cytostatic and cytotoxic effects of oridonin (Rubescenin), a diterpenoid from Rabdosia rubescens, on tumor cells of different lineage. *Int. J. Oncol., 26*, 579–588.

Choi, J. Y., Yoo, J. Y., Kwak, H. S., Nam, B. U., & Lee, J. (2005). Role of polymeric stabilizers for drug nanocrystal dispersions. *Current Applied Physics, 5*, 472–474.

Clement, M. A., Pugh, W., & Parikh, I. (1992). Tissue distribution and plasma clearance of a novel microcrystal-encapsulated flurbiprofen formulation. *Pharmacologist, 34*, 204–211.

Fujita, T., Takeda, Y., Sun, H. D., Minami, Y., Marunaka, T., & Takeda, S. (1988). Cytotoxic and antitumor activities of Rabdosia diterpenoids. *Planta. Med., 54*, 414–417.

Hecq, J., Deleers, M., Fanara, D., Vranckx, H., & Amighi, K. (2005). Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. *Int. J. Pharm., 299*, 167–177.

Ikezoe, T., Chen, S. S., Tong, X. J., Heber, D., Taguchi, H., & Koeffler, H. P. (2003). Oridonin induces growth inhibition and apoptosis of a variety of human cancer cells. *Int. J. Oncol., 23*, 1187–1193.

Jacobs, C., Kayser, O., & Muller, R. H. (2000). Nanosuspensions as a new approach for the formulation for the poorly soluble drug tarazepide. *Inter. J. Pharm., 196*, 161–164.

Keck, C. M., & Muller, R. H. (2006). Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *Eur. J. Pharm. Biopharm., 6*, 3–16.

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

Kocbek, P., Baumgartner, S., & Kristl, J. (2006). Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. *Inter. J. Pharm., 312*, 179–186.

Krause, K. P., & Muller, R. H. (2001). Production and characterisation of highly concentrated nanosuspensions by high pressure homogenisation. *Int. J. Pharm., 214*, 21–24.

Lee, J., Lee, S. J., Choi, J. Y., Yoo, J. Y., & Ahn, C. H. (2005). Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion. *Eur. J. Pharm. Sci., 24*, 441–449.

Liversidge, E. M., Liversidge, G. G., & Cooper E. R. (2003). Nanosizing: A formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci., 18*, 113–120.

Liversidge, G. G., & Cundy, K. C. (1995). Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int. J. Pharm., 125*, 91–97.

Li, Y. Ch., Dong, L., Jia, A., Chang, X. M., & Xue, H. (2006). Preparation and characterization of solid lipid nanoparticles loaded traditional Chinese medicine. *Inter. J. Bio. Macro., 38*, 296–299.

Liu, Ch. J., & Zhao, Zh. H. (1998). Research progress of oridonin. *Chin. Pharm. J., 33*, 577–581.

Moschwitzera, J., Achleitnerb, G., Pomperb, H., & Muller, R. H. (2004). Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. *Eur. J. Pharm. Biopharm., 58*, 615–619.

Muller, R. H., Becker, R., Kruss, B., & Peters, K. (1999). Pharmaceutical nanosuspensions for medicament administration as systems with increased saturation solubility and rate of solution. U.S. Patent No. 5,858,410.

Muller, R. H., & Jacobs, C. (2002). Buparvaquone mucoadhesive nanosuspension: preparation, optimisation and long-term stability. *Int. J. Pharm., 237*, 151–161.

Muller, R. H., Jacobs, C., & Kayser, O. (2001). Nanosuspensions as particulate drug formulations in therapy Rationale for development and what we can expect for the Future. *Adv. Drug. Deliv. Rev., 47*, 3–19.

Osawa, K., Yasuda, H., Maruyama, T., Morita, H., Takeya, K., & Itokawa, H. (1994). Antibacterial trichorabdal diterpenes from Rabdosia trichocarpa. *Phytochemistry, 36*, 1287–1291.

Patravale, V. B., Date, A. A., & Kulkarni, R. M. (2004). Nanosuspensions: A promising drug delivery strategy. *J. Pharm. Pharmaco., 56*, 827–840.

Peters, K., Leitzke, S., Diederichs, J. E., Borner, K., Hahn, H., Muller, R. H., et al. (2000). Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. *J. Antimicrob. Chemother., 45*, 77–83.

Siostrom, B., Kronberg, B., & Carlfors, J. (1993). A method for the preparation of submicron particles of sparingly water-soluble drugs by precipitation in oil-in-water emulsions: 1. Influence of emulsification and surfactant concentration. *J. Pharm. Sic., 82*, 579–583.

White, R. D., Wong, J., Kipp, J., Barber, T., Glosson, J., Kerzee, J., et al. (2003). Pre-clinical evaluation of itraconazole nanosuspension for intravenous injection. *Toxicol. Sci., 72*, 51–59.

Xing, J., Zhang, D. R., & Tan, T. W. (2007). Studies on the oridonin-loaded poly(d,l-lactic acid) nanoparticles in vitro and in vivo. *Int. J. Bio. Macro., 40*, 153–158.

Zhang, D. R., & Ren, T. C. (2003). Pharmaceutical progress of oridonin. *Chin. Pharm. J., 38*, 817–820.

Zhang, D. R., Tan, T. W., & Gao, L. (2007). Preparation of oridonin-loaded SLN and studies on it in vitro and in vivo. *Nanotech, 17*, 5821–5828.

Zhang, J. X., Han, Q. B., Zhao, A. H., & Sun, H. D. (2003). Diterpenoids from Isodon japonica. *Fitoterapia, 74*, 435–438.

Zhang, Y., Wang, J., Lou, L. G., Zhang, T. M., Hou, J. W., & Xin, W. J. (1999). Scavenging effect of oridonin on active oxygen free radicals. *Henan. Med. Res., 8*, 100–104.

Zhang, Y. B., Kou, X., Lu, J. Sh., & Wang, G. H. (2001). Research on the oridonin-β-cyclodextrin complex. *Chinese Traditional and Herbal Drugs, 24*, 131–132.