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Research paper

Particle size control and the interactions between drug and stabilizers in an amorphous nanosuspension system

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ABSTRACT

Nanosuspension, especially the amorphous type of that, is an effective way to improve the dissolution properties of poorly water-soluble drugs. Amorphous nanosuspensions of resveratrol were prepared here by a precipitation method without homogenization or ultrasonication step attached. The interactions between drug and stabilizers were revealed by infrared (IR) spectra and differential scanning calorimetry (DSC) and interpreted with the assistance of modulation of their conformational dynamics. When nonionic polymer PVA was used as a stabilizer, PVP was found to be capable of controlling the size of secondary particles by inducing the aggregation and agglomeration of primary nanoparticles, which can be attributed to the formation of hydrogen bonds. This method could be applicable to controlling the size of nanoparticles or change their surface state in similar nanoparticle systems.

1. Introduction

An active pharmaceutical ingredient (API) that demonstrates poor water solubility and low oral bioavailability will likely face significant challenges during the drug development process. Among the factors influencing dissolution properties of a given API molecule, particle size is of considerable importance. The significant increase in surface energy and area obtained by particle size reduction greatly improves the dissolution velocity and saturation solubility, hence the bioavailability.

Nanosuspension, which is defined as colloidal dispersion of nano-sized particles of water-insoluble drug(s), has obtained great success in addressing the dissolution problems [1,2]. Unlike other nanoparticle-based drug delivery systems, in which the drug is confined to a cavity surrounded by a polymer membrane or physically dispersed in a matrix, nanosuspension involves pure drug nanoparticles dispersed in a liquid medium without any membrane or matrix material. Nevertheless, some suitable stabilizers (surfactants or polymer stabilizers) are usually added to inhibit the aggregation of drug nanoparticles [3,4]. The interactions between drug and stabilizers are believed to exist in nanosuspension systems but difficult to be revealed because the stabilizers would cause significant interference, although raw materials of stabilizers were usually taken as control in current practice.

The solid drug particles in nanosuspensions can exist in two forms: crystalline state and amorphous state. Crystalline nanoparticles, also called nanocrystals, are usually prepared by top-down approaches, including wet milling [5,6] and high pressure homogenization [7]. Amorphous nanosuspensions are often produced by bottom–up techniques, among which, antisolvent precipitation [6–12] are typically used. Nanocrystals could also be formed in a bottom–up process in case the crystallization is not inhibited effectively [13]. Accordingly, polymer stabilizers that can interfere with crystallization in a competitive manner, are indispensable for amorphous nanosuspensions. In most cases, ultrasonication [6,8,9] or high-speed stirring [10–12] should be incorporated in a precipitation procedure to minimize the particle size, which adds to the complexity and difficulty of scale-up.

Amorphous solids, because they exhibit a higher energy state than crystalline solids, often have higher solubility and dissolution velocity. Both the amorphous and crystalline particles suffer from unsatisfactory stability due to Ostwald ripening. However, for an

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amorphous suspension, Ostwald ripening can be inhibited by incorporating a small amount of additive [14]. Therefore, amorphous nanosuspension should be extensively concerned.

Particle size is one of the most important properties of any nanosuspension system, because it greatly affected the physical stabilities and biological performances as well as the dissolution properties. The influence of particle size on the in vivo tissue distribution [15–17] and mucoadhesion [18,19] has been demonstrated in nanoparticle drug delivery systems, which also can be reasonably expected in nanosuspension systems. During the preparation of nanosuspension, particle size control can be accomplished by adjusting the time and/or strength of milling or homogenization for "top–down" approach, but it is still a hurdle for "bottom–up" approach.

Here we prepared amorphous nanosuspensions of resveratrol using a simple precipitation method, and investigated the size control of drug solid particles using a pair of polymers PVA/PVP. Hydrogen bond induced aggregation and agglomeration could be a possible mechanism for the variation of particle size.

The drug we used here is trans-resveratrol, a poorly water-soluble stilbenoid, which is found in the skin of grapes and is well known for the French paradoxon [20]. We have reported the preparation of amorphous resveratrol nanosuspension [12], and its crystalline counterpart has also been reported [21]. Here we placed emphasis on the simplification of preparation method, the solid state confirmation and its related change in spectra, the interaction between drug and stabilizers, and most importantly, the particle size control of this nanosuspension system.

2. Materials and methods

2.1. Materials

The commercial trans-resveratrol (98.9% pure, needle crystal) was purchased from Tianjin Jianfeng Nature Product R&D Co., Ltd. (Tianjin China). Absolute ethanol, polyvinyl alcohol (PVA-0588, Mw 24500–29500), Tween-80 and poloxamer 188 (P188) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China); Polyvinylpyrrolidone (PVP, Kollidon 17PF, Mw7000–11000) was obtained from BASF Corporation of Germany (Local Agent in Shanghai, China). All the chemical reagents were used as received.

2.2. Preparation of nanosuspensions

Nanosuspensions of resveratrol were produced using precipitation method in the dark. In short, the drug was dissolved in ethanol at a concentration of 10 mg/mL and then 1 mL of the solution was rapidly injected into 10 mL of aqueous solution containing stabilizer(s) at room temperature. A magnetic stirrer was used (200 rpm, 15 min) during precipitation to provide efficient mixing of the solvent–antisolvent.

To control the particle size, a nanosuspension, in which a solution containing 0.4% (w/v) Tween and 0.2% (w/v) PVA was used as the precipitating liquid, was prepared at first and labeled as F_0. Then a 0.8% (w/v) PVP solution was added to change the particle size. Respectively, 2.5 mL and 5.0 mL of the PVP solution were added into 10 mL of the nanosuspension F_0 to give F_1 and F_2, which were determined for further characterization.

2.3. Morphology and particle size determination

The morphological examination of the nanoparticles was performed using a 200 kV Transmission Electron Microscope (TEM) (Tecnai G20, FEI Company, Hillsboro, Oregon, USA). The prepared nanosuspension was dropped on carbon-coated copper grids and dried at room temperature for viewing. For particle sizes analysis, nanosuspensions were diluted and then determined by dynamic light scattering (DLS) method using a nanoparticle analyser (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK) at room temperature.

2.4. Powder X-ray diffraction (PXRD)

PXRD was used to monitor the crystalline state of lyophilized F_0~F_2. The PXRD patterns were measured using a Bruker D8 Advance diffractometer (Bruker AXS GmbH) with Cu as the anode material operated at a voltage of 40 kV and current of 40 mA. The instrument was operated with a scanning rate of 1°/min and a scanning range of the 2θ from the initial angle 4° to the final angle 80°.

2.5. Thermal analysis using differential scanning calorimetry (DSC)

The DSC analysis of lyophilized samples was carried out using differential scanning calorimetry (DSC Q2000, TA Instruments Inc., New Castle, USA) over a temperature range of 40 °C to 300 °C at a heating rate of 10 °C per minute. Measurements were performed under a dry nitrogen atmosphere and an empty pan was used as reference. Besides pure resveratrol and nanosuspensions (F_0~F_2), an aqueous solution of Tween&PVA&PVP at the same concentration as F_1 was lyophilized and analyzed.

2.6. Fourier transform infrared spectroscopy (FT-IR)

FT-IR absorption spectra of lyophilized samples were recorded on a Thermo Nicolet Avatar 370 spectrophotometer at frequencies ranging from 400 to 4000 cm^{-1} with a 2 cm^{-1} resolution. Five samples, the same as that analyzed with DSC, were respectively mixed with KBr and pressed into pellets before determination.

2.7. Computational method

Firstly, resveratrol, PVA and PVP were simplified as three molecular models, namely I, II and III respectively (Fig. S1, see Supplementary materials). The geometries of the molecular models were constructed and fully optimized using density functional theory (DFT) at the ωB97X-D/6-31++g** level [22,23]. Then, the structures of complexes were built according to the calculated geometries of the drug and stabilizers and optimized at the same level of theory. The binding energy was calculated using the equation as follow:

$$\Delta E = E(\text{drug} - \text{macromolecule complex}) - E(\text{drug}) - E(\text{macromolecule})$$

All calculations were performed using the Gaussian 09 program [24].

3. Results and discussion

3.1. Optimization of stabilizers

Resveratrol nanosuspensions here were obtained by a precipitation method which is in the category of "bottom–up" approach. When ethanol diffused to the aqueous phase containing stabilizer, the dissolved drug begins to precipitate and form nanoparticles due to the size limitation effect afforded by stabilizer. Fig. 1 shows the resveratrol nanosuspensions respectively stabilized by four different stabilizers. Surfactants are more effective than hydrophilic polymers in reducing Gibbs free energy theoretically. However,