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Preparation and characterization of spironolactone nanoparticles by antisolvent precipitation

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ABSTRACT

Due to low aqueous solubility and slow dissolution rate, spironolactone, a synthetic steroid diuretic, has a low and variable oral bioavailability. Nanoparticles were thus prepared by antisolvent precipitation in this work for accelerating dissolution of this kind of poorly water-soluble drugs. Effects of surfactant type/concentration and feed drug concentration on the precipitated particle size were evaluated. It was found that introduction of spironolactone solution in N-methyl-2-pyrrolidone (NMP) to the antisolvent water can produce the particles in the submicron range with hydroxypropyl methylcellulose (HPMC) as the stabilizer. The particle size decreased with the increase of HPMC concentration from 0 to 0.125% (w/v), further increase of which did not affect the size significantly. Increasing feed drug concentration from 10 to 100 mg/ml resulted in the particle size decrease. In comparison with raw drug, the chemical structure of nanosized spironolactone was not changed but the crystallinity was reduced. Dissolution of spironolactone nanoparticles in 0.1 M HCl was 2.59 times faster than raw drugs in 60 min.

1. Introduction

Spironolactone is a synthetic steroidal diuretic to treat a series of diseases, such as edema, cirrhosis of the liver, and hypokalemia. Due to its low aqueous solubility and slow dissolution rate, spironolactone exhibits a variable and incomplete oral bioavailability (Levy, 1962; Clarke et al., 1977). Reducing drug particles size is an effective and widely used approach to speed up dissolution by enlarging the effective surface area. According to Noyes–Whitney equation, the dissolution rate is proportional to the surface area exposed to the dissolution medium (Kesisoglou et al., 2007). It has been found that 2.21 μm spironolactone particles-made tablet had a significantly greater oral bioavailability than 78.8 μm drug particles-made tablet (McInnes et al., 1982). Thereby, to guarantee oral bioavailability, it is required that more than 90% of spironolactone particles be less than 10 μm (China Pharmacopeia, 2005). In recent years, reducing the drug particles size further down to the submicron range has been gaining much attention, since a much higher oral bioavailability could be achieved due to the further enlarged surface area in comparison with micronized drugs (Liversidge and Conzentino, 1995; El-Shabouri, 2002; Merisko-Liversidge et al., 2003; Langguth et al., 2005). In addition, saturation solubility can also be increased for drugs in the submicron range, which would further increase dissolution rate and oral bioavailability. Other methods, such as complexation with cyclodextrin, lyophilization and solid dispersion, have also been explored to accelerate dissolution of spironolactone (Losowsky, 1991; Yusuff et al., 1991; Soliman et al., 1997; Hodges et al., 2006; Uchino et al., 2007).

The purpose of this work is to produce spironolactone nanoparticles for dissolution rate enhancement. Generally, drug nanoparticles can be produced by the "breaking-down" or "building-up" technique. The former involves the diminution of the coarse large drug particles down to the submicron range by virtue of various milling or high pressure homogenization techniques; the latter is actually to build the drug nanoparticles starting from the molecules, which includes the antisolvent precipitation technique, supercritical fluid technology (rapid expansion of supercritical solution, RESS and gas antisolvent precipitation, GAS) and spray-freezing/evaporation into liquid (Rabinow, 2004; Keck and Muller, 2006; Kesisoglou et al., 2007). For spironolactone, submicron particles have been produced by high pressure homogenization (Langguth et al., 2005) and emulsion/solvent diffusion technique (El-Shabouri, 2002). In this work, we prepared spironolactone nanoparticles by antisolvent precipitation technique, which has not been reported in the literature, to our knowledge. For this technique, an organic drug solution is introduced to the antisol-

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vent under rapid mixing, which generates high supersaturation and thereby fast nucleation rate leading to production of submicron particles. This technique has the advantage of low cost, effective energy consumption and easy scaling-up (Keck and Muller, 2006). In this work, water was used as the antisolvent for the precipitation of spironolactone. The appropriate solvent and stabilizer were screened by investigating the size of the precipitated particles. Dynamic laser light scattering (DLLS) technique was used to measure the particle size. Effects of surfactant type and concentration, drug concentration on particle size were investigated. Morphology and thermal behavior of the raw spironolactone and precipitated particles were examined by scanning electronic microscopy (SEM) and differential scanning calorimetry (DSC), respectively. Also, FTIR was used to investigate the drug chemical structure. Saturation solubility was tested by Higuchi method using high performance liquid chromatography (HPLC). Finally, dissolution profiles of raw spironolactone and precipitated drug nanoparticles were studied by USP XXV type II (paddle) method.

2. Materials and methods

2.1. Materials

Spironolactone was purchased from Sigma. Hydroxypropyl methyl cellulose (HPMC, M_n 86,000) and polyvinylpyrrolidone K30 (PVPK30) were provided by Fluka. Tween 80, sodium dodecyl sulfate (SDS), polyvinyl alcohol (PVA, mol. wt. 30,000–70,000) and Pluronic F-127 were from Sigma. 1-Methyl-2-pyrrolidone (NMP) was supplied by Fluka. Other solvents, such as ethanol, methanol, isopropanol (IPA) and acetone, were obtained from Sigma. HPLC grade acetonitrile was from Sigma.

2.2. Preparation of spironolactone nanoparticles

Spironolactone nanoparticles were produced by antisolvent precipitation technique. Briefly, a certain amount of drug was completely dissolved in water-miscible solvent. The obtained drug solution was then injected into the water containing the stabilizer under stirring at 1000 rpm. Precipitation of solid drug particles occurred immediately upon mixing. The suspension was centrifuged at 20,000 rpm for 10 min and washed twice with deionized water. The particle pellet was oven-dried at 65 °C for 1 day.

2.3. Size measurement

Size of the formed drug particles was measured immediately after precipitation by dynamic laser light scattering (Nano-Zetasizer, Malvern). Before analysis, the drug suspension was diluted by deionized water to 0.2 mg/ml. The measurement was done in triplicate and size d_{90} and d_{50} was reported.

2.4. Morphology

Morphology of drug particles was visualized by a field emission scanning electron microscope (FESEM, JEOL JSM-6700F). The drug particles were sputter coated with gold for 40 s before observation.

2.5. Saturation solubility

Saturation solubility of raw spironolactone and precipitated drug nanoparticles was determined by Higuchi method. In brief, excess amount of drug powders was put into 2 ml deionized water in a capped vial and stirred for 72 h. The suspension was then filtered (pore size: 0.22 μm) and the filtrate was directly injected to HPLC system (Agilent 1100), which was equipped with the Agilent Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm). The mobile phase, composed of 50% acetonitrile and 50% Millipore water (v/v), was delivered at 0.8 ml/min. The drug was detected at 238 nm and the retention time of the drug was ~12 min. The drug concentration in the filtrate is its saturation solubility. The experiment was conducted in triplicate.

2.6. FTIR

Bruker Vertex 70 FTIR Emission Spectrometer was used to record the FTIR spectrum of the drugs from 400 to 4000 cm^{-1}. The sample was grounded with KBr and pressed to a suitable-size disk for measurement.

2.7. DSC

Differential scanning calorimetry (DSC) was conducted on Diamond DSC Calorimeter (PerkinElmer). The samples were equilibrated at 20 °C for half hour and then heated to 220 °C at 10 °C/ml in a N_2 atmosphere.

2.8. Dissolution test

Dissolution of drugs was performed according to the USP XXV type II (paddle) method (VK 7010 dissolution apparatus, VARIAN). The rotation speed of paddle was set to be 100 rpm and the bath temperature was kept at 36.5 ± 0.5 °C. Ten milligrams of drug powder was put into the vessel containing 900 ml 0.1 M HCl solution. At specific intervals, 0.5 ml aliquot of the dissolution medium was sampled, filtered (pore size: 0.22 μm) and directly injected to the HPLC system. The analysis conditions were the same as described above.

3. Results and discussion

3.1. Preparation of spironolactone nanoparticles

Spironolactone nanoparticles were prepared by antisolvent precipitation in this work. For this technique, introduction of the drug solution to the antisolvent generates high supersaturation. This results in fast nucleation rate and produces a large number of nuclei, which reduces the solute mass for subsequent growth. Submicron particles can thus be obtained provided that the growth of nucleating crystals can be arrested by the stabilizer (surfactant or polymer) through steric or electrostatic mechanism (Matteucci et al., 2006). For hydrophobic drugs like spironolactone, water is most commonly used as the antisolvent. In terms of the solvent, it is beneficial if it can solubilize the drug in large amount and possesses a fast diffusion rate to the antisolvent water; while the stabilizer needs to have good affinity for drug particles and possess a fast diffusion rate and effective adsorption onto the drug particles surface in the water–solvent mixture. Therefore, solvent–stabilizer pair is crucial to obtain submicron particles. So far, identification of an appropriate solvent–stabilizer pair is empirical. In this work, among the water-miscible solvent candidates ethanol, methanol, NMP, acetone and IPA, NMP was selected due to its highest capacity to solubilize spironolactone (>100 mg/ml). The appropriate stabilizer was thus screened by trial and error from PVA, PVPK30, HPMC, Pluronic F-127, SDS and Tween 80. Preliminary results (SEM images not shown) showed that, spironolactone particles, precipitated by mixing organic drug solution with water containing HPMC, were in the submicron range. This indicates that the hydrophobic part of HPMC has good affinity for drug particles and thereby is able to provide effective steric barrier against growth. In addition, HPMC molecules have sufficient rate of diffusion to and adsorption onto the drug particle surface in the NMP–water mixture.

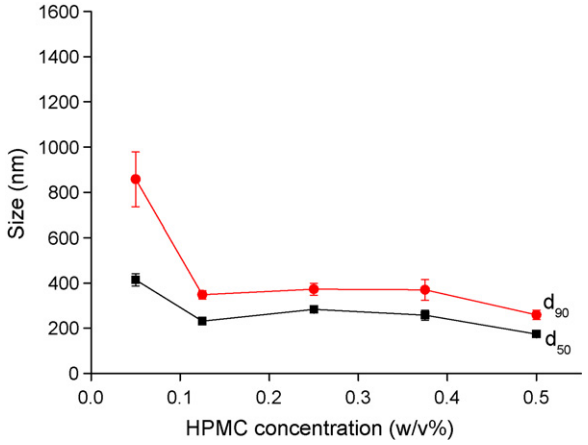

Fig. 1. Effect of HPMC concentration on the particles size (drug concentration: 50 mg/ml; NMP/water volume ratio: 1/10).

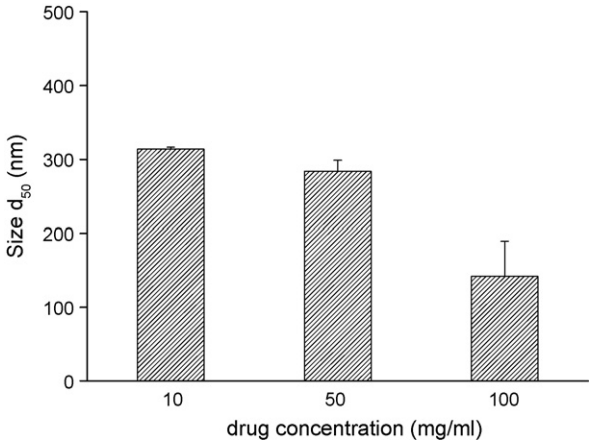

Fig. 2. Effect of feed drug concentration on the particles size (NMP/water volume ratio: 1/10; HPMC concentration: 0.05, 0.25 and 0.5% for 10, 50 and 100 mg/ml drug solution, respectively).

Effect of HPMC concentration on the spironolactone particle size is shown in Fig. 1. Without HPMC, the precipitated particles were several microns in size (estimated from the SEM image, Fig. 4b). When 0.05% (w/v) HPMC was added in the water, the average size d_{50} of achieved particles was dramatically decreased to 413.7 ± 27.2 nm indicating that HPMC is effective to provide steric stabilization and arrest the particle growth. Increasing the HPMC concentration to 0.125% resulted in a further size reduction to 231 ± 1 nm; after that, the particles size was not significantly influenced by increasing HPMC concentration, which indicates that the drug particle surface is already sufficiently enveloped by the HPMC molecules. The d_{90} size of all the particles precipitated with different concentration of HPMC (0.05–0.5%) as stabilizer were less than 1 μm as shown in Fig. 1, which indicates a narrow size distribution. In addition, d_{90} of the precipitated particles exhibited the same dependence on the HPMC concentration as d_{50} did.

Feed drug concentration determines supersaturation, which influences the precipitated particle size in two ways: on one hand, the higher supersaturation leads to a faster nucleation rate and thereby smaller particles; on the other hand, higher supersaturation favors particles growth by promoting condensation and/or coagulation (Matteucci et al., 2006). As can be seen from Fig. 2, with the increase of drug concentration from 10 to 100 mg/ml, the average particles size decreased from ~300 nm to below 200 nm. This means that the increased potential of particle growth, caused by higher supersaturation at high feed drug concentration, is effectively inhibited by the stabilizer HPMC.

Potential particle growth after precipitation is a common phenomenon for most of the drugs. The size reported above was obtained by measuring the drug particles immediately after precipitation. We also examined the particles at 10 min after precipitation and found to be several microns. However, by checking the SEM images of a small drop of a very diluted suspension, it was found that the particles at 0 min after precipitation are discrete with a size of several hundred nanometers (Fig. 3a), while the particles after 10 min are several micron-large aggregates, which is composed of a large number of individual submicron particles (Fig. 3b). It can thus be concluded that HPMC is effective in arresting the particle growth, but may not be very effective to prevent aggregation.

3.2. Morphology

Morphology of raw spironolactone and precipitated drug particles after oven-drying is shown in Fig. 4. It can be seen that raw drug particles exhibit irregular shape and a broad size distribution (Fig. 4a). Precipitated drug without the aid of any stabilizer during the preparation process shows cuboid structure with several microns in size (Fig. 4b). The drug particles precipitated with the HPMC as stabilizer show spherical or twisted cuboid shape in whole and the size ranges from several to over 10 μm (Fig. 4c). Under high magnification, it can be clearly observed that these agglomerates or particle assemblies are composed of a large number of individual nanoparticles with a size of ~300 nm (Fig. 4d). Aggregation of the precipitated nanoparticles, on one hand, would retard the drug dissolution due to the reduced effective surface area (Liversidge and Cundy, 1995); while on the other hand, it may be beneficial to subsequent powder handling, e.g. granulation process.

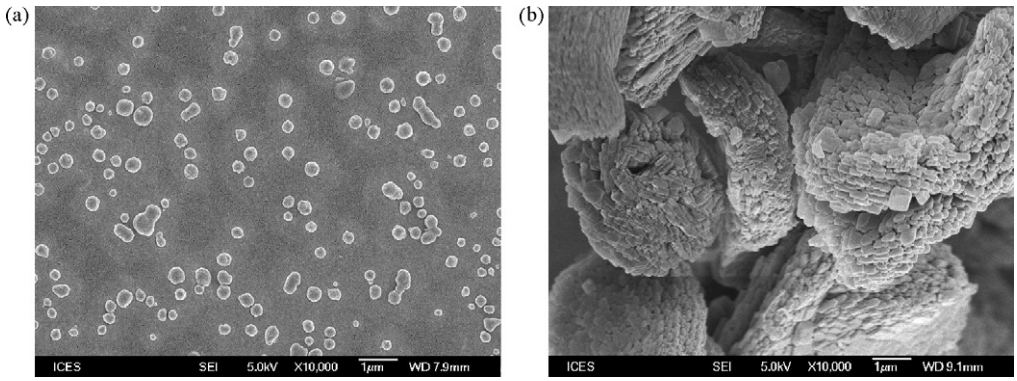

Fig. 3. SEM image of diluted particles suspension at (a) 0 min and (b) 10 min after precipitation (drug concentration: 50 mg/ml; HPMC concentration: 0.25%; NMP/water volume ratio: 1/10).

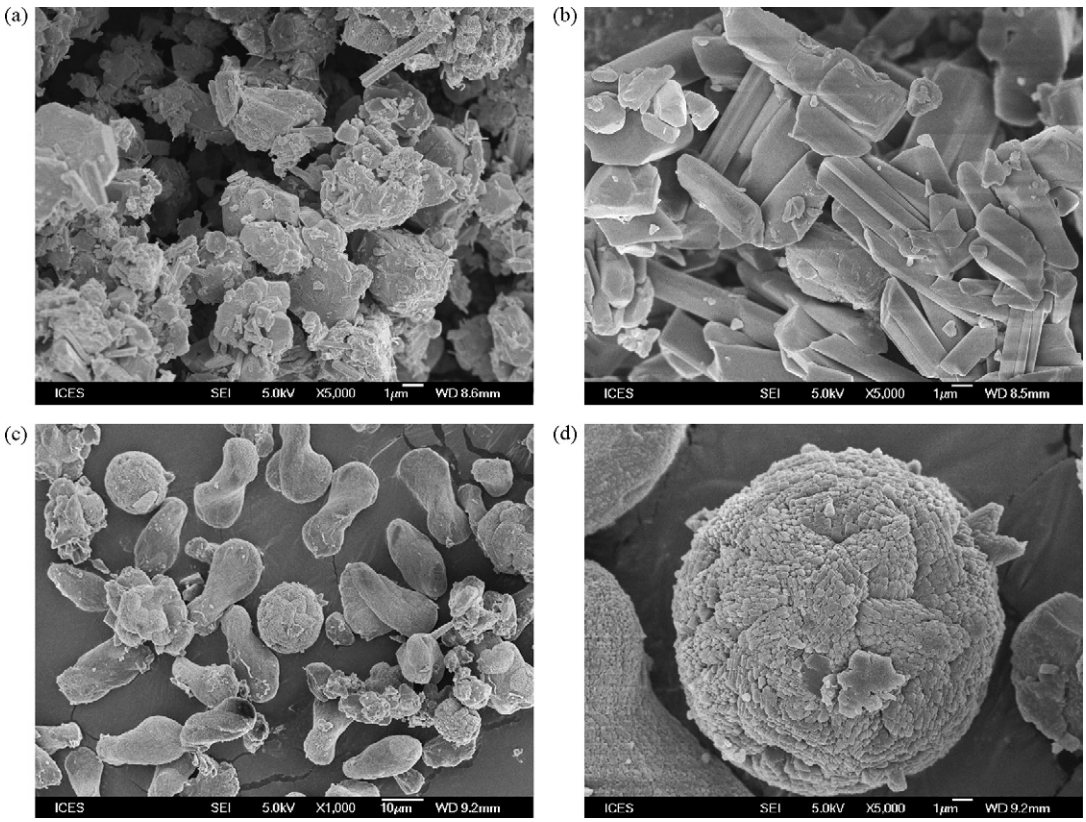

Fig. 4. SEM images of (a) raw drug particles, (b) precipitated drug particles without surfactant, (c) precipitated drug nanoparticles using HPMC and (d) drug nanoparticles (c) under high magnification.

3.3. FTIR

Raw spironolactone and precipitated nanoparticles exhibited same FTIR spectrum as shown in Fig. 5, which demonstrates that the chemical structure of the drug is not changed before and after the precipitation process.

3.4. DSC

The physical state of raw spironolactone and drug nanoparticles was examined by DSC and their thermograms are shown in Fig. 6. Raw spironolactone exhibited a melting point at 207.9 °C with fusion enthalpy of 54.1 J/g. After being precipitated as nanoparticles, its melting point and fusion enthalpy was decreased to 203.4 °C and 44.5 J/g, respectively, indicating reduced crystallinity. This phenomenon can be explained by the fact that under fast nucleation rate, the drug solute lacks sufficient time to incorporate into the growing crystal lattice accurately to form perfect crystals (Rabinow, 2004). It is worthwhile to note that there is an exothermic peak at 164.6 °C (circle in the figure) for nanosized spironolactone, which may be due to the recrystallization upon heating of the drug (Agafonov et al., 1991; Berbenni et al., 1999; Espeau et al., 2007). This again demonstrates the reduced crystallinity of submicron spironolactone particles compared to raw drug.

3.5. Saturation solubility and dissolution

Saturation solubility of raw spironolactone and precipitated nanoparticles at room temperature was 12.77 ± 0.65 and 15.98 ± 1.14 μg/ml, respectively. Reducing the particle size down to the submicron range increases the saturation solubility of spironolactone by 25%. Dissolution profiles of raw spironolactone and precipitated nanoparticles are illustrated in Fig. 7. To preclude the

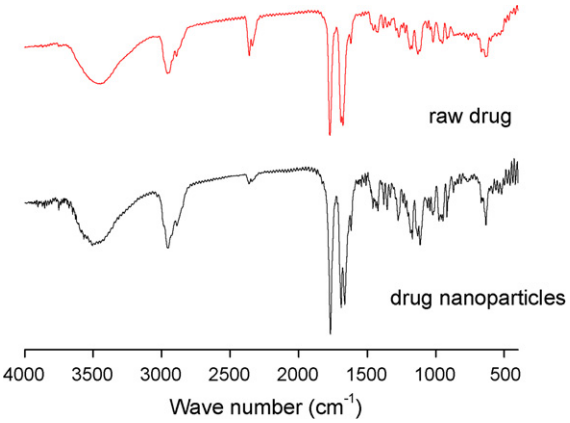

Fig. 5. FTIR of pristine and precipitated drug particles.

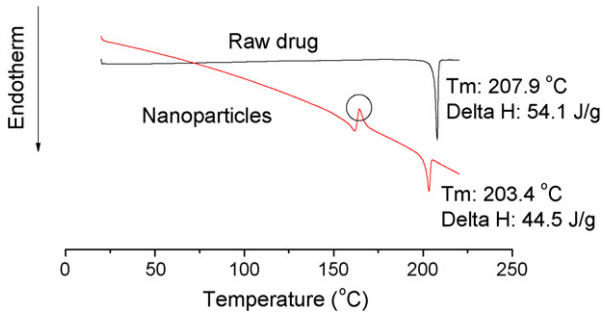

Fig. 6. DSC thermograms of raw spironolactone and drug nanoparticles.

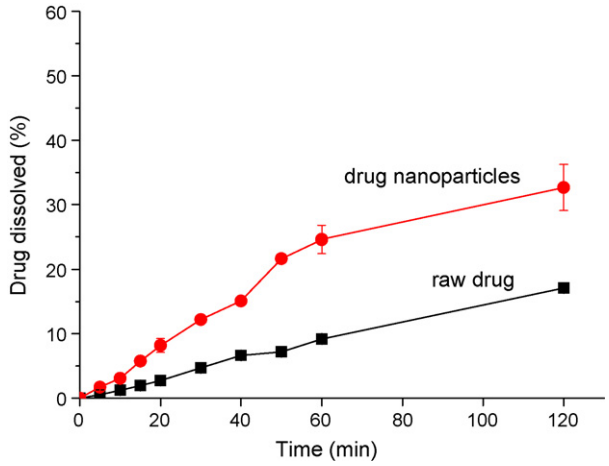

Fig. 7. Dissolution profile of raw spironolactone and drug nanoparticles.

influence of stabilizer, HPMC was intentionally washed off during the preparation process. As can be seen, 9.2% of the raw spironolactone was dissolved in 60 min; while in the same period, 24.6% of the drug nanoparticles was dissolved. The dissolution rate of spironolactone nanoparticles is 2.67 times that of raw drugs. According to Noyes–Whitney equation, the solid dissolution rate is directly proportional to its surface area exposed to the dissolution medium. The accelerated dissolution for spironolactone nanoparticles could thus be mainly ascribed to their greater surface area in comparison with raw drug. Increased saturation solubility and decreased crystallinity of nanosized spironolactone could also contribute as well. It is noted that the extent of dissolution rate enhancement is not as high as expected from the extent of size reduction (several microns down to ~300 nm). The reason could be due to the aggregation of nanoparticles, which reduced the effective surface area for dissolution. In the period of 60–120 min, the released raw drug was 7.9%, which is nearly same as the drug content (8.0%) dissolved from spironolactone nanoparticles. This indicates that the acceleration of drug dissolution for nanoparticles occurs mostly in the first 60 min; after that, the dissolution has no significant difference from raw drugs.

4. Conclusion

Antisolvent precipitation technique was employed to producing nanoparticles of spironolactone, a poorly water-soluble drug, for the enhancement of solubility and dissolution velocity. With water as antisolvent, NMP and HPMC were found to be the suitable solvent and stabilizer, respectively, to produce submicron particles. As HPMC concentration increased, the particle size was decreased until a threshold was reached. Increasing feed drug concentration results in size reduction in the range of 10–100 mg/ml. The precipitated spironolactone nanoparticles had the same chemical structure with raw drug, but the crystallinity was reduced. Spironolactone nanoparticles exhibited significantly faster drug dissolution compared with raw drug. Further work to evaluate the effect of stabilizer and/or excipients on preventing aggregation of nanoparticles, both in the precipitation and drying process and achieving long term-stability is on-going. Antisolvent precipitation can thus be a simple and effective approach to produce submicron particles of poorly water-soluble drugs.

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