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A continuous and highly effective static mixing process for antisolvent precipitation of nanoparticles of poorly water-soluble drugs

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ABSTRACT

Rapid and homogeneous mixing of the solvent and antisolvent is critical to achieve submicron drug particles by antisolvent precipitation technique. This work aims to develop a continuous and highly effective static mixing process for antisolvent precipitation of nanoparticles of poorly water-soluble drugs with spironolactone as a model drug. Continuous antisolvent production of drug nanoparticles was carried out with a SMV DN25 static mixer comprising 6–18 mixing elements. The total flow rate ranged from 1.0 to 3.0 L/min while the flow rate ratio of solvent to antisolvent was maintained at 1:9. It is found that only 6 mixing elements were sufficient to precipitate the particles in the submicron range. Increasing the number of elements would further reduce the precipitated particle size. Increasing flow rate from 1.0 to 3.0 L/min did not further reduce the particle size, while higher drug concentrations led to particle size increase. XRD and SEM results demonstrated that the freshly precipitated drug nanoparticles are in the amorphous state, which would, in presence of the mixture of solvent and antisolvent, change to crystalline form in short time. The lyophilized spironolactone nanoparticles with lactose as lyoprotectant possessed good redispersibility and showed 6.6 and 3.3 times faster dissolution rate than that of lyophilized raw drug formulation in 5 and 10 min, respectively. The developed static mixing process exhibits high potential for continuous and large-scale antisolvent precipitation of submicron drug particles.

1. Introduction

Reduction of size of drug particles to the submicron range, by either "top down" or "bottom up" principle, is an effective approach to enhance the dissolution rate of the poorly water-soluble drugs and increase oral bioavailability (Jia et al., 2002; Merisko-Liversidge et al., 2003; Rabinow, 2004; Keck and Muller, 2006; Kesisoglou et al., 2007). In the pharmaceutical industry, the drug nanoparticles are mainly produced by the "top down" principle and the applied techniques include milling and high pressure homogenization (Jacobs and Muller, 2002; Keck and Muller, 2006). In contrast, the "bottom up" technique, such as antisolvent precipitation, supercritical fluid technology, spray freezing into liquid, etc., is seldom employed. In comparison with milling and high pressure homogenization, in fact, some "bottom up" techniques, for example antisolvent precipitation are quite simple, cost effective and easy for scaling-up (Horn and Rieger, 2001; Rogers et al., 2004). For antisolvent precipitation of poorly water-soluble drug nanoparticles, the drug is first dissolved in a solvent, which is rapidly mixed with a solvent-miscible antisolvent (e.g. water). High supersaturation is thus generated due to the fast diffusion of the solvent into the antisolvent, which thereby results in fast nucleation rate leading to production of submicron particles (Horn and Rieger, 2001; Matteucci et al., 2006). Instant and homogeneous mixing of the solvent and the antisolvent is crucial to achieve the particles in the submicron range. In this respect, the traditional stirred tank reactor is apparently not suitable for a continuous antisolvent precipitation of drug nanoparticles at a large scale. Highly efficient mixing equipment is needed to realize the rapid and homogeneous mixing of two miscible fluids, i.e. solvent and antisolvent. In the literature, mixing chambers (Horn and Rieger, 2001), Y or T mixers (Choi et al., 2005; Kim and Kim, 2006), static mixers (Gassmann et al., 1994; Douroumis and Fahr, 2006; Douroumis et al., 2008) and rotating packed bed (Chen et al., 2006) have been reported for this purpose. The process is quite similar: upon being pumped to the mixing equipments, the solvent and antisolvent are mixed rapidly inside and are flushed out continuously as a precipitated nanoparticles suspension stream. By connecting an industry-scale

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spray dryer to the outlet of the mixing equipment and addition of some aggregation–inhibition excipients (lactose, mannitol, etc.) in the antisolvent, a fully continuous process can be achieved from the starting materials, i.e. the solvent and antisolvent, to the final solid redispersible drug powders (Gassmann et al., 1994; Horn and Rieger, 2001). Therefore, selection of suitable mixing equipment is of great importance for such a continuous antisolvent precipitation process. Among the aforementioned mixing equipments, static mixer is of great attraction due to the advantages offered, such as no energy needed except pumping, low equipment cost, compact space requirement and high effectiveness of instant and homogeneous mixing of miscible fluids (Thakur et al., 2003). A static mixer is composed of a series of identical and motionless elements, which possess specific structure and are inserted into pipes, columns or reactors. Once the two flows are pumped into the pipes, columns or reactors, these elements are able to redistribute fluid in the radial and tangential directions to realize the rapid and homogeneous mixing. Gassmann first reported the use of static mixers for production of drug hydrosols in 1994 (Gassmann et al., 1994). Very recently, Douroumis applied static mixing for antisolvent precipitation of drug nanoparticles (Douroumis and Fahr, 2006; Douroumis et al., 2008). However, both studies have not included a detailed investigation into the effects of various process parameters on the precipitated drug particles, such as flow rate and drug concentration, which are actually very important in terms of productivity and effectiveness to achieve submicron particles from the eyes of scaling-up. Also, the efficiency of the developed static mixing process is not so high, as a relatively high number of mixing elements were needed to achieve the nanosized drug particles even at low drug concentration.

Spironolactone is a steroidal diuretic for treatment of edema, cirrhosis of the liver, and hypokalemia, etc. Belonging to the Class II drug, spironolactone possesses low aqueous solubility and slow dissolution rate, which results in a variable and incomplete oral bioavailability (Levy, 1962; Clarke et al., 1977). Various approaches have been attempted to accelerate its dissolution rate and thereby enhance oral bioavailability, such as complexation with cyclodextrin, lyophilization and solid dispersion (Yusuff et al., 1991; Soliman et al., 1997; Hodges et al., 2006; Uchino et al., 2007). As an effective and universal strategy, reduction of the spironolactone particles size to the micrometer, especially to the nanometer range, has gained much attention both in the industry and research (McInnes et al., 1982; EI-Shabouri, 2002; Langguth et al., 2005; China Pharmacopeia, 2005; Dong et al., 2009).

Our previous work described a batch production of spironolactone nanoparticles of poorly water-soluble drugs by antisolvent precipitation technique (Dong et al., 2009). In this work, we aimed to develop a continuous process for antisolvent precipitation of nanoparticles of poorly water-soluble drugs in large scale. For this purpose, the SMV type static mixer designed for turbulent flow was selected to be the mixing equipment and spironolactone was used as a model drug. Effects of various process parameters, such as flow rate, drug concentration and number of mixing elements on the size of the precipitated drug particles, were investigated. Dynamic laser light scattering (DLLS) technique was used to measure the particle size. Morphology of the particles was examined by the scanning electronic microscopy (SEM). Crystallinity of particles were studied by X-ray diffraction (XRD). Finally, the drug dissolution behavior was examined by USP XXV type II (paddle) method.

2. Materials and methods

2.1. Materials

Spironolactone was purchased from Wuhan Hezhong Biochemical Manufacture Co., Ltd., China. Hydroxylpropylmethyl cellulose (HPMC), sodium dodecyl sulfate (SDS) and lactose were obtained from Sigma. Acetone was supplied by Fisher Scientific.

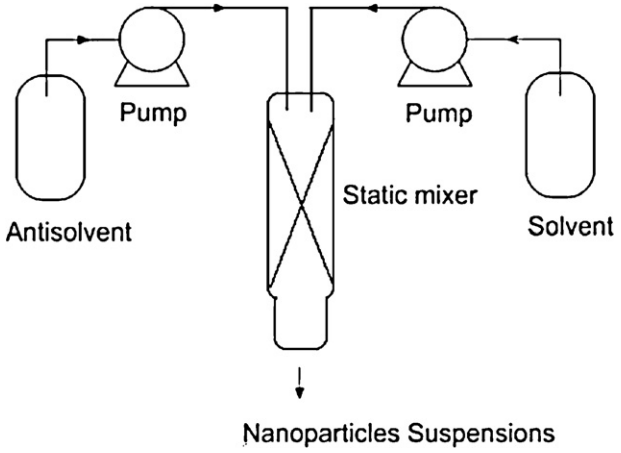

Fig. 1. Antisolvent precipitation of spironolactone nanoparticles by static mixers.

2.2. Antisolvent precipitation of drug nanoparticles by static mixing

Static mixing technique was used in this work to produce spironolactone nanoparticles by antisolvent precipitation. The SMV DN25 static mixers for turbulent flow were supplied by Sulzer Chemtech, Switzerland. Each mixing element had a size of 25 mm and six elements were welded together by being offset 90° to each other forming one segment, which was inserted to a glass tube for application. The process of antisolvent precipitation of spironolactone nanoparticles by static mixing is illustrated in Fig. 1. The solvent, i.e. drug solution in acetone (50–200 mg/ml) and the antisolvent water (with 0.55–2.2 mg/ml HPMC and 0.55–2.2 mg/ml SDS) were pumped at a flow rate of 0.1–0.3 and 0.9–2.7 L/min, respectively; their flow rate ratio was maintained at 1:9. The diameters of the nozzles for solvent and antisolvent were 0.5 and 1.5 mm, respectively. The velocity of the solvent and antisolvent were thus kept same and ranged from 8.5 to 25.5 m/s. Upon being pumped into the mixers, the solvent and antisolvent were mixed instantly and flushed out continuously as milky drug suspensions at a flow rate of 1–3 L/min. To obtain the solid powders, 9.9 mg/ml lactose was included in the antisolvent water. The freshly formed drug suspensions were immediately frozen using the liquid nitrogen and then lyophilized for 5 days.

2.3. Particle size measurement

Dynamic laser light scattering technique (Nano-Zetasizer, Malvern) was used to measure the size of the freshly precipitated drug particles and the reconstituted solid powders. To reduce the effect of particles interaction, the drug particles concentration was adjusted by diluting with deionized water to approximately to 0.2 mg/ml. The measurement was done in triplicate and the *z*-average size was reported.

2.4. Morphology

Morphology of the freshly precipitated drug particles and the lyophilized nanoparticles formulation was observed by a field emission scanning electron microscope (FESEM, JEOL JSM-6700F). For the freshly prepared particles, a drop of suspension was dripped onto a double-sided carbon tape attached to a SEM stud and oven

dried at 60 °C for 12 h. The particles were sputter coated with gold for 40 s before visualization.

2.5. X-ray diffraction

X-ray diffraction measurements were carried out using a D8-ADVANCE (BRUKER) X-ray diffractometer from 5 to 40 (2θ) at a step of 0.017° using Cu Kα radiation.

2.6. Dissolution measurement

Drug dissolution measurements of lyophilized nanoparticle formulation were performed according to the USP XXV type II (paddle) method (VK 7010, VARIAN). To preclude effects of excipients, raw drug was also lyophilized together with lactose, HPMC and SDS in the same ratio with nanoparticles formulation. The rotation speed of paddle and the bath temperature were set to be 100 rpm and 37 °C, respectively. An equivalent of 25 mg drug (~80 mg lyophilized powders) was placed into the vessel containing 900 ml of 0.1 M HCl as the dissolution medium. At 5, 10, 15, 20, 30, 40 and 60 min, 0.5 ml aliquot of the dissolution medium was taken out, filtered (pore size: 0.22 μm) and directly injected to the HPLC system (Agilent 1100) for drug concentration analysis. The column used was Agilent Eclipse XDB-C18 column (5 μm, 4.6 mm × 250 mm) and the mobile phase was the mixture of 50% acetonitrile and 50% Millipore water (v/v) with a flow rate of 0.8 ml/min. The drug was detected at wavelength 238 nm and the retention time of the drug was ~12 min. The experiment was conducted in triplicate.

3. Results and discussion

3.1. Antisolvent precipitation of spironolactone nanoparticles using static mixers

Antisolvent precipitation is a simple but quite effective approach to produce nanoparticles of poorly water-soluble drugs by mixing the drug solution (solvent) and the antisolvent water. A continuous and large-scale process may be developed provided that the selected mixing equipment is able to realize the instant and homogeneous mixing of the solvent and antisolvent. In this work, a SMV static mixer designed for turbulent flow was used for the rapid and intensive mixing of spironolactone solution in acetone and the antisolvent water. The velocity of the two injected flow was kept same and ranged from 8.5 to 25.5 m/s by adjusting the volumetric flow rate.

Keeping the ratio of the flow rate of solvent to antisolvent at 1:9, the precipitated particles had a *z*-average size of 482 ± 56, 448 ± 9 and 480 ± 1 nm, when the total flow rate was 1.0, 2.0 and 3.0 L/min, respectively (Fig. 2). It can be seen that, at a total flow rate 1.0 L/min, the submicron particles were already successfully achieved and increasing the flow rate did not further reduce the particle size. At a total flow rate of 1.0 L/min, i.e. 0.1 and 0.9 L/min for the solvent and antisolvent respectively, the solvent and antisolvent were both injected to the static mixer at 8.5 m/s. The corresponding Reynolds number Re was calculated to be 1.09×10^4 and 1.26×10^4, respectively according to the following equation:

$$Re = \nu \cdot d/\mu$$

ν: flow velocity, 8.5 m/s for both the solvent and antisolvent; d: nozzle diameter, 0.5 and 1.5 mm for solvent and antisolvent, respectively; μ: kinematic viscosity, 0.39×10^{-6} m^2/s (acetone) and 1.01×10^{-6} m^2/s (water) were taken here for solvent and antisolvent, respectively.

It has been reported that increase in turbulence, i.e. higher Re, would lead to smaller particles (Matteucci et al., 2006). In our case, however, both the solvent and antisolvent are already in fully developed turbulent flow conditions at a total flow rate of 1.0 L/min. Further increase of the extent of turbulence (Re) would not pose a significant influence on the mixing efficiency. Therefore, the size of the precipitated particles is not further reduced by increasing the flow rate of the solvent and antisolvent.

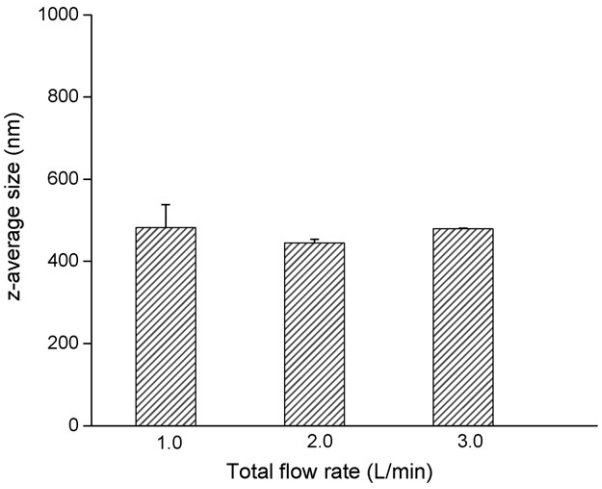

Fig. 2. Effect of flow rate (L/min) on the size of precipitated particles.

Drug concentration in the mixture of solvent/antisolvent affects the size of precipitated particles in opposing ways: high drug concentration leads to high supersaturation, which results in a faster nucleation rate and thereby smaller particles; however, high supersaturation also speeds up agglomeration promoting production of bigger particles (Matteucci et al., 2006). Fig. 3 illustrates the effect of spironolactone concentration in the mixture of acetone/water on the size of precipitated particles. As can be seen, increasing the drug concentration from 5 to 10 and 20 mg/ml resulted in a size increase of the precipitated particles from 482 ± 56 to 733 ± 98 and 1233 ± 42 nm, respectively, which indicates that agglomeration prevails over nucleation at high drug concentrations. The results shows that the developed static mixing process in this work is able to achieve submicron drug particles at drug concentration as high as 10 mg/ml, which is quite cost effective in terms of the reduction of the solvent used and also beneficial for the post-treatment, such as spray drying or freeze-drying.

Size of the precipitated particles is significantly affected by the effectiveness of static mixing, which depends largely on the

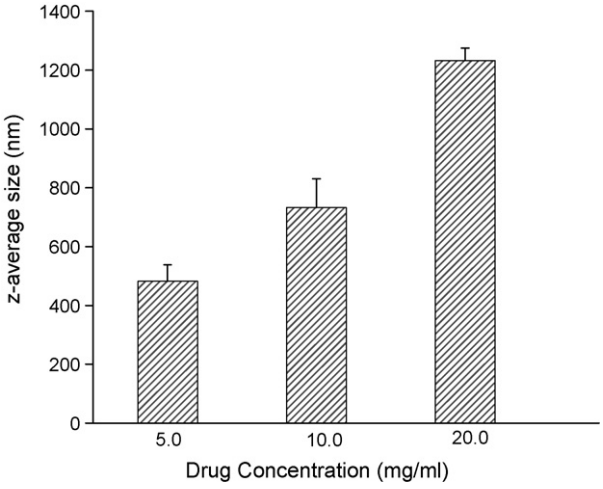

Fig. 3. Effect of drug concentration on the size of precipitated particles.

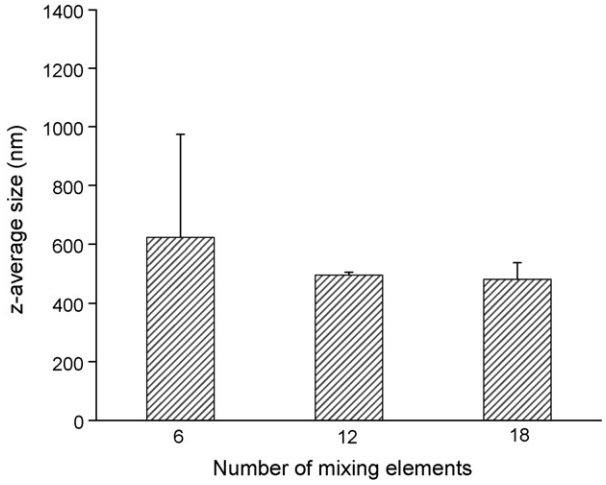

Fig. 4. Effect of the number of mixing elements on the size of precipitated particles.

structure and number of the mixing elements (Thakur et al., 2003). Douroumis et al. has shown that using the SMX type static mixers designed for laminar flow, 30 mixing elements were required to achieve submicron drug particles by antisolvent precipitation technique (Douroumis and Fahr, 2006; Douroumis et al., 2008). In our work, as shown in Fig. 4, however, only 6 mixing elements were already sufficient to achieve the drug particles in the submicron range, although the size distribution was wide. Increase in the number of mixing elements to 12 resulted in a decrease of size and size distribution. Further increase to 18 mixing elements, however, did not lead to a further size reduction indicating the effectiveness of the static mixing has probably reached a plateau.

In comparison with previous reports (Douroumis and Fahr, 2006; Douroumis et al., 2008), the developed static mixing process in this work exhibits high efficiency in that the submicron drug particles can be successfully achieved even at high drug concentration (i.e. 10 mg/ml) using fewer mixing elements. The reasons could be: (1) the static mixer used in our work was SMV model designed for turbulent flow instead of SMX typical intended for laminar flow and (2) both the solvent and antisolvent were injected into the static mixer in fully developed turbulent flow conditions. Therefore, a rapid and intensive turbulent mixing is achieved and the mixing efficiency is sufficiently high enough to achieve the drug particles in the submicron range.

3.2. Morphology

Morphologies of raw spironolactone, drug nanoparticles obtained by oven-drying of a drop of fresh precipitates and lyophilized nanoparticles formulation are shown in Fig. 5. Raw drug particles exhibit irregular shape and a broad size distribution (Fig. 5a). The precipitated particles achieved by oven-drying a drop of suspension had a cuboidal shape with length of ~500 nm and width of ~100 nm (Fig. 5b). Lyophilized spironolactone nanoparticles formulation exhibited a continuous and amorphous structure, into which the drug nanoparticles are interdispersed (Fig. 5c). Lyophilized formulation (Fig. 5c) was reconstituted with water and the entrapped drug particles were found to be spherical in shape with ~200 nm (Fig. 5d) indicating an amorphous state of spironolactone in the lyophilized nanoparticle formulation.

3.3. XRD

Lyophilized spironolactone nanoparticles formulation and raw drug formulation both contained a drug content of ~33.3% and lactose content of ~60%; the surfactants HPMC and SDS accounted for the remaining proportion equally. As shown in Fig. 6, the drug spironolactone (Fig. 6a), the excipient lactose (Fig. 6b) and the surfactant SDS (Fig. 6c) are highly crystalline; while HPMC is in amorphous state. Lyophilized raw drug formulation (Fig. 6e)

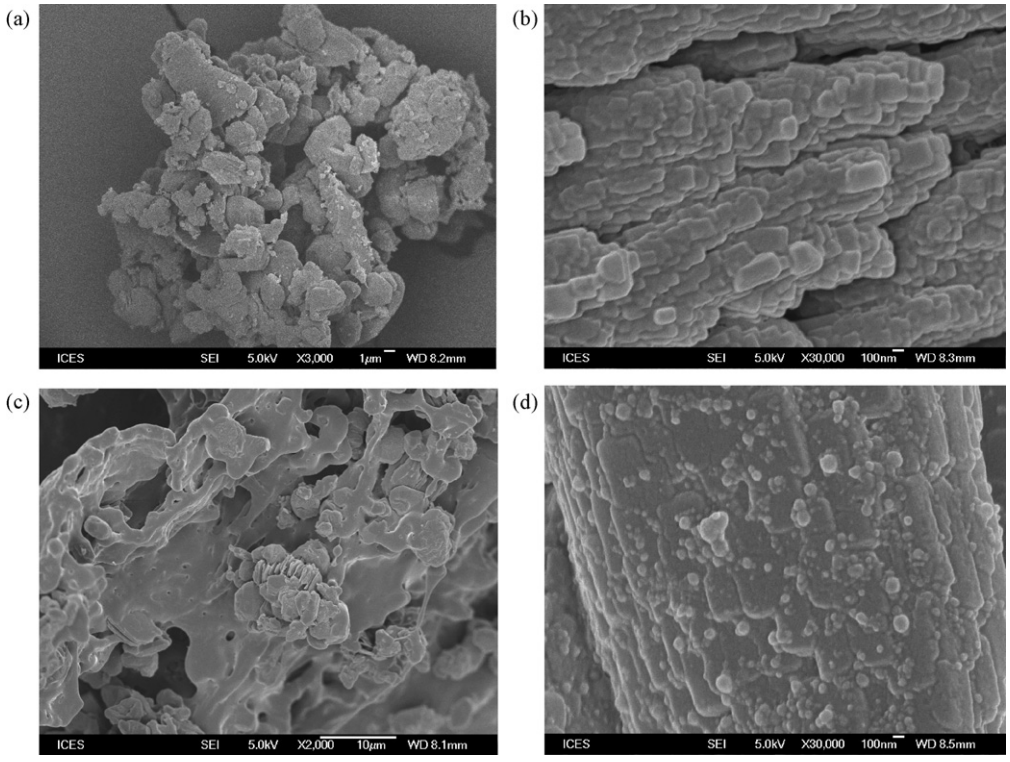

Fig. 5. SEM images of (a) raw spironolactone (b) drug nanoparticles achieved by oven-drying of a drop of freshly precipitated suspensions (c) lyophilized spironolactone nanoparticles formulation and (d) reconstitution of (c).

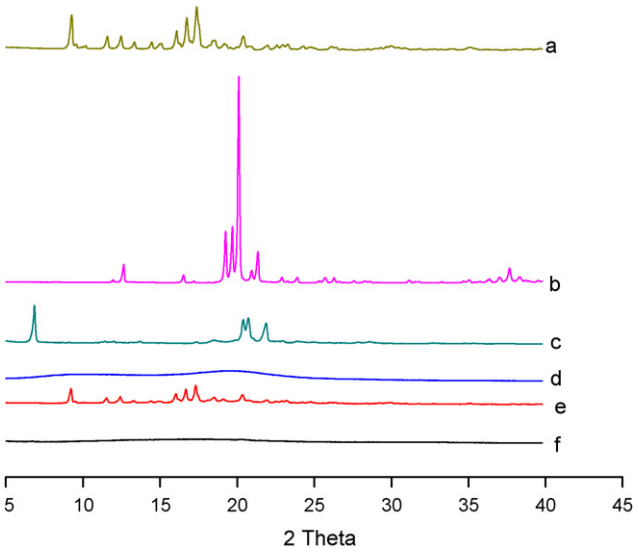

Fig. 6. XRD spectrum of (a) spironolactone, (b) lactose, (c) SDS, (d) HPMC, (e) lyophilized raw drug formulation and (f) lyophilized drug nanoparticles formulation.

exhibited the same XRD spectrum as the raw drug (Fig. 6a), which indicates that the lactose is desiccated out in the amorphous state with the removal of water during lyophilization process, while the drug spironolactone still remains crystalline. The peak of SDS was not detected in the spectrum perhaps due to its low content in the formulation. For lyophilized nanoparticles formulation (Fig. 6f), no peak can be found indicating both of the drug and lactose are amorphous. A comparative study was performed and showed that, if the freshly formed spironolactone nanoparticles suspensions were left alone for several minutes, instead of immediately being immersed in the liquid nitrogen, and then lyophilization, the obtained drug was in crystalline state. Together with the results of SEM, it is thus concluded that, the freshly precipitated spironolactone are in the amorphous form, which would change to the crystalline state if left on its own.

3.4. Redispersibility

In comparison with microparticles, nanosized drug particles possess many specific advantageous features, such as enlarged surface area, increased saturation solubility, adhesiveness to the gut wall and so on, which finally result in an increased and constant oral bioavailability. To fully benefit from these advantages, however, one prerequisite is that these nanoparticles, after being formulated, should still be disintegrated to individual nanoparticles upon contact with the physiological fluids (Keck and Muller, 2006). Some sugars (lactose, mannitol, etc.) are thus generally added to the nanoparticles suspension during the spray drying or freeze-drying process for this purpose (Abdelwahed et al., 2006; Chaubal and Popescu, 2008). The principle is that the dried nanoparticles are interdispersed in the continuous solid matrix of the sugars to prevent aggregation. In this work, lactose was used as the lyoprotectant for freeze-drying of the spironolactone nanoparticles. It can be seen from Fig. 7 that the size of the reconstituted particles was measured to be still in the submicron range indicating a good redispersibility of the developed lyophilized nanoparticles formulation.

3.5. Dissolution

Both the lyophilized spironolactone nanoparticles formulation and the control, i.e. lyophilized raw drug formulation contained a drug loading of ~33%, lactose ~60%, HPMC ~3.3% and SDS

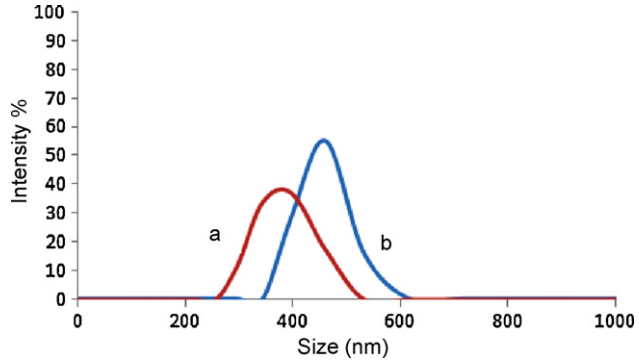

Fig. 7. Size distribution of (a) freshly precipitated drug nanoparticles and (b) reconstituted lyophilized nanoparticles formulation.

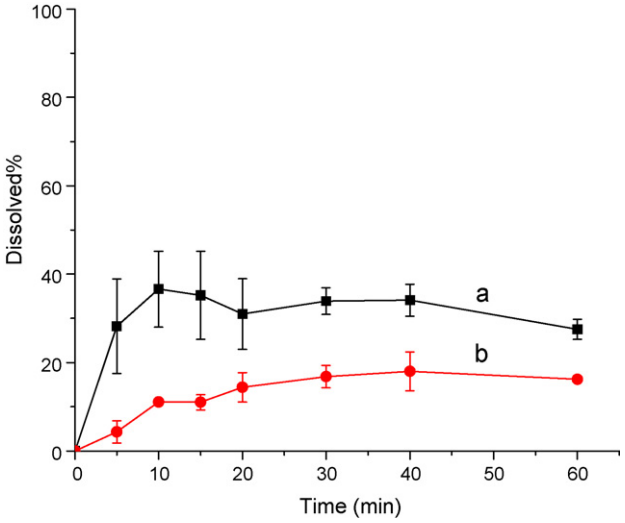

Fig. 8. Dissolution profile of (a) lyophilized spironolactone nanoparticles formulation and (b) lyophilized raw drug formulation.

~3.3%, respectively. Their dissolution profiles are shown in Fig. 8. In 5 min, 4.3% and 28.2% of the drug was dissolved from the raw spironolactone and nanosized drug formulations, respectively; spironolactone nanoparticles exhibits 6.6 times faster in dissolution rate than raw drugs. In 10 min, the dissolution rate of nanoparticles formulation was 3.3 times that of the raw drug formulation; the quantities dissolved were 11.1% and 36.6%, respectively. In addition, the dissolution of drug from lyophilized nanoparticles formulation has reached peak in 10 min in view of the dissolution curve in 60 min. The result demonstrates that the dissolution of lyophilized spironolactone nanoparticles formulation is much faster than raw drug formulation. According to Noyes-Whitney equation, the drug dissolution rate is linearly proportional to the surface area exposed to the dissolution medium. The accelerated dissolution for lyophilized spironolactone nanoparticles formulation could thus be mainly ascribed to their greater surface area in comparison with lyophilized raw drug formulation. The amorphous state of the lyophilized nanoparticles would also help accelerate the drug dissolution rate as well (Lindfors et al., 2007).

4. Conclusions

A continuous and large-scale static mixing process was developed in this work to produce spironolactone nanoparticles by antisolvent precipitation. Effects of flow rate, drug concentration and the number of mixing elements were studied. At a total flow rate of 1.0–3.0 L/min, ~500 nm particles were successfully

obtained, which was not significantly influenced by increasing the flow rate. The size of precipitated particles is reduced with the number of mixing elements and 6 mixing elements are sufficient to precipitate the particles in the nanometer range. Increasing the drug concentration would increase the particles size and the submicron particles can be obtained at 10 mg/ml. Lyophilized nanoparticles formulation exhibited much faster dissolution rate in comparison with the raw drug formulations. It is noteworthy to point out that storage conditions have significant influences on the stability of lyophilized nanoparticles formulation, such as crystallinity, redispersibility, dissolution, etc. This will be studied in our future work.

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