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RESEARCH ARTICLE

Preparation of stable micron-sized crystalline irbesartan particles for the enhancement of dissolution rate

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Abstract

Purpose: In this study, micron-sized crystalline drug particles of irbesartan (IBS) were prepared to improve its stability and dissolution rate.

Method: The approach to crystalline particles was based on the liquid precipitation process by which the amorphous particles were prepared. Pharmaceutical acceptable additives were used as the crystallization agent to convert the amorphous drug into crystalline particles. High pressure homogenization (HPH) process has been employed to reduce the size of the crystalline particles, and the micron-sized particles were obtained by the freeze-drying process.

Results: Different additives show different influences on the polymorphic form of IBS. Polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) were effective in stabilizing amorphous particles instead of converting amorphous drug into crystalline particles, while poloxamer407 (F127) and tween80 (T80) could convert the amorphous drug into crystalline particles. T80 was also effective in controlling the particle size than that of F127. After HPH, crystalline particles with an average of 0.8 μm were obtained. The freeze-dried micron-sized crystalline particles exhibited significantly enhanced *in vitro* dissolution rate when compared to the raw drug. SEM, FT-IR, XRD, DSC and dissolution rate studies indicated that the micron-sized particles were stable during 6 months storage.

Conclusion: The preparation of micron-sized crystalline drug particles is an effective way to improve the stability and dissolution rate of irbesartan.

Keywords: Irbesartan, crystallization, micron-sized particles, poorly water-soluble drug, dissolution rate

Introduction

The fact that 40% or more of the newly developed drugs are poorly water-soluble presents serious obstacles to the development of their applications in drug formulation and delivery[1]. In the case of the biopharmaceutics classification systems (BCS) class II drugs which have high permeabilities through biomembranes, the dissolution rate in the gastrointestinal tract limits the bioavailability[2]. In the process of overcoming issues involving solubility and dissolution rate, reducing particle size to micro/nano-range has emerged as an effective option to enhance the dissolution rate and bioavailability[3–7].

Irbesartan (IBS) is an angiotensin II receptor antagonist, which was used for the treatment of cardiovascular diseases such as hypertension, cardiac insufficiency, cardiac arrhythmia[8,9]. However, as one of the BCS class II drugs, the therapeutic effects of IBS are discounted by its poor aqueous solubility, which results in poor oral absorption and bioavailability. To solve this problem, several approaches such as formation of solid dispersions[10], anti-solvent precipitation[11], formation of β-cyclodextrin complex[12], have been employed to enhance the dissolution rate of IBS. Nevertheless, IBS prepared by those methods was in amorphous state. Application of amorphous drug is theoretically

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an ideal approach to enhance the dissolution rate[7,13,14], because the amorphous particles could increase the solubility and the surface area available for dissolution[15]. However, amorphous particles are not thermally stable, and there is an uncontrollable tendency for the amorphous particles to recrystallize the more stable crystalline forms. Uncontrollable recrystallization process is unwanted, and it could result in the aggregation of particles, consequently, neutralizing the solubility and dissolution advantage offered by amorphous drug particles[16,17].

IBS has four polymorph forms, which include form A, B, C and the amorphous form[18–20]. The raw IBS and commercial available IBS tablets are form A. The aim of this study was to prepare micron-sized crystalline particles of form A to improve the dissolution rate and stability of IBS. Our route to crystalline particles was based on the liquid precipitation process by which the amorphous particles were prepared, and pharmaceutical acceptable additives were used as the crystallization agent to convert the amorphous drug into crystalline particles. The size of the obtained crystalline particles was further reduced by the high pressure homogenization (HPH) process. The effects of crystallization time, temperature and HPH process parameters on the polymorphs form and particle size were investigated. The products were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD), differential scanning calorimetry (DSC), specific surface area, dissolution and stability testing.

Experimental section

Materials

The raw IBS (form A, purity >99.4%) was purchased from Liuhe Xiuzheng Pharmaceutical Co. Ltd. (Jilin, China). Tween80 (T80) was obtained from Beijing Chemical Reagents Company (Beijing, China). Poloxamer407 (F127) and polyvinylpyrrolidone (PVP) were supplied by Beijing Baierdi Biopharmaceutical Co. Ltd. (Beijing, China). Hydroxypropyl methylcellulose (HPMC) was provided by Zhejiang Zhongwei Pharmaceutical Co. Ltd. (Zhejiang, China). Methanol and hydrochloric acid (HCl) were of analytical grade and obtained commercially from Beijing Chemical Reagents Company. Acetonitrile was HPLC grade and provided by FisherChemical (NJ, USA). Deionized water was purified with a Hitech-K Flow Water Purification System (Hitech Instruments Co. Ltd., Shanghai, China).

Methods

The raw IBS was dissolved in methanol with the concentration of 1 wt%, and the solution was filtered through a 0.45-μm nylon membrane to remove the possible impurities. In the typical experiment, 50 mL of the drug solution was poured rapidly into 1000 mL deionized water by means of stirring (2000 rpm). After stirring for 30 s, amorphous IBS suspension was formed. And then, 100 mg of additive was dissolved in the suspension. The suspension was stirred for 10 min, and amorphous drug was converted into crystalline particles. Then, the suspension was filtered and washed with deionized water. The resultant filter paste was dispersed in water and processed by HPH (AH110D, Engineering INC, Italy). The homogenized suspension was freeze-dried using a model LT-105 lyophilizer (Martin Christ, Osterode, Germany) at a shelf temperature of −40°C and a pressure below 0.5 mbar for 48 h to yield the dry powder.

Particle size and morphology

The particles surface appearance and shape were analyzed by SEM, JSM-6360LV (JEOL, Japan). Samples were prepared by finely spreading a glass slide with a small drop of the suspension and draining the liquid with filter paper, and then coated with a fine gold layer in an argon atmosphere and observed by SEM. The volume particle size of the drug particles was determined using a laser diffractometer (Malvern; ZETASIZER-3000HS). The sample was diluted by deionized water and sonicated to create a homogenous suspension. All the samples were analyzed in triplicate.

Specific surface area

The specific surface area of the samples was measured using N_2 adsorption and implemented by Surface Area Analyzer ASAP 2010-M (Micromeritics Instrument Corporation, Norcross, GA). Before measuring, all the sample powders were degassed for at least 4 h at room temperature and measured using a six point pressure profile ranging from 0.05 to 0.30 psia. The calculation was based on the BET equation.

Chemical composition and physical characteristics

FT-IR analysis was carried out to evaluate the molecular states of the samples. FT-IR spectra were recorded with a Nicolet model 8700 spectrometer (Nicolet Instrument Corporation, Madison, WI) in the range 525–4000 cm^{-1}. Samples were diluted with KBr mixing powder at 1% and pressed to obtain self-supporting disks.

XRD was performed using Cu Kα radiation which was generated at 30 mA and 40 kV (XRD-6000, Shimadzu Inc., Japan). The scanning speed was 5°/min from 3° to 40° with a step size of 0.02°.

DSC was employed to detect the crystallinity of micron-sized particles (Q200, TA). The heating rate was 10°C/min, and a dry nitrogen purge of 20 mL/min was used. Calibration of the instrument with respect to temperature and enthalpy was achieved using high purity standard of indium. The samples used for XRD and DSC analysis were prepared by lyophilization of the filter paste with a model LT-105 lyophilizer (Martin Christ, Germany).

To investigate the stability of the dry powder, the freeze-dried dry powder was sealed in a polyethylene bag under room conditions for more than 6 months. After

storage, the FT-IR, XRD and DSC analysis as well as the dissolution study was conducted.

In vitro dissolution study

In vitro dissolution study was carried out following the USP Apparatus 2 (paddle) method (D-800LS, Tianjin, CN). The paddle speed was 50 rpm, and the dissolution medium was 0.1 mol/L HCl which was maintained at 37.0 ± 0.5°C. The powder (75 mg) was added into vessels containing 900 mL of the dissolution medium, and 2 mL samples were withdrawn at specific intervals (5, 10, 15, 20, 30, 40, 50, 60, 90, and 120 min). In the meantime, fresh medium (2 mL) was added to keep constant volume. The samples were filtered using a 0.22-μm filter. The concentrations of the drugs were measured by the high performance liquid chromatography (HPLC) system. Each sample was analyzed in triplicate.

HPLC analysis

The HPLC system consisted of a Waters 2695 Separations Module and a Waters 2996 Photodiode Array Detector (Waters Corporation, Miford, MA) using a Waters Sunfire™ C_{18}, reverse-phase column (150 mm × 4.6 mm i.d., 5 μm particle size). The mobile phase consisting of 52% of 0.02 mol/L potassium dihydrogen orthophosphate (pH 2.6) and 48% of acetonitrile at a flow rate of 1.0 mL/min, and the effluent was monitored at wavelength 245 nm. Data acquisition and evaluation were performed with Waters Empower2 Chromatography Data software.

Results and discussion

Preparation of the crystalline particles

Figure 1A presents the SEM images of amorphous IBS drug particles obtained by liquid precipitation. The particles had a spherical shape and an average size of 780 nm. To convert the amorphous drug into crystalline, we investigated the possibility of converting the amorphous IBS into crystalline without additives and with different pharmaceutical additives (PVP, HPMC, F127 and T80). Without additives, after 6 h, amorphous IBS particles recrystallize into crystalline form, which was confirmed by XRD (Figure 2). However, the particles were fused seriously (Figure 1B). Therefore, it is necessary to select suitable additives to improve the crystallization rate as well as suppress random fusion of drug particles. Using PVP or HPMC as the additives, the results of XRD demonstrate that IBS still maintained as amorphous state after 6 h (Figure 2), indicating both of

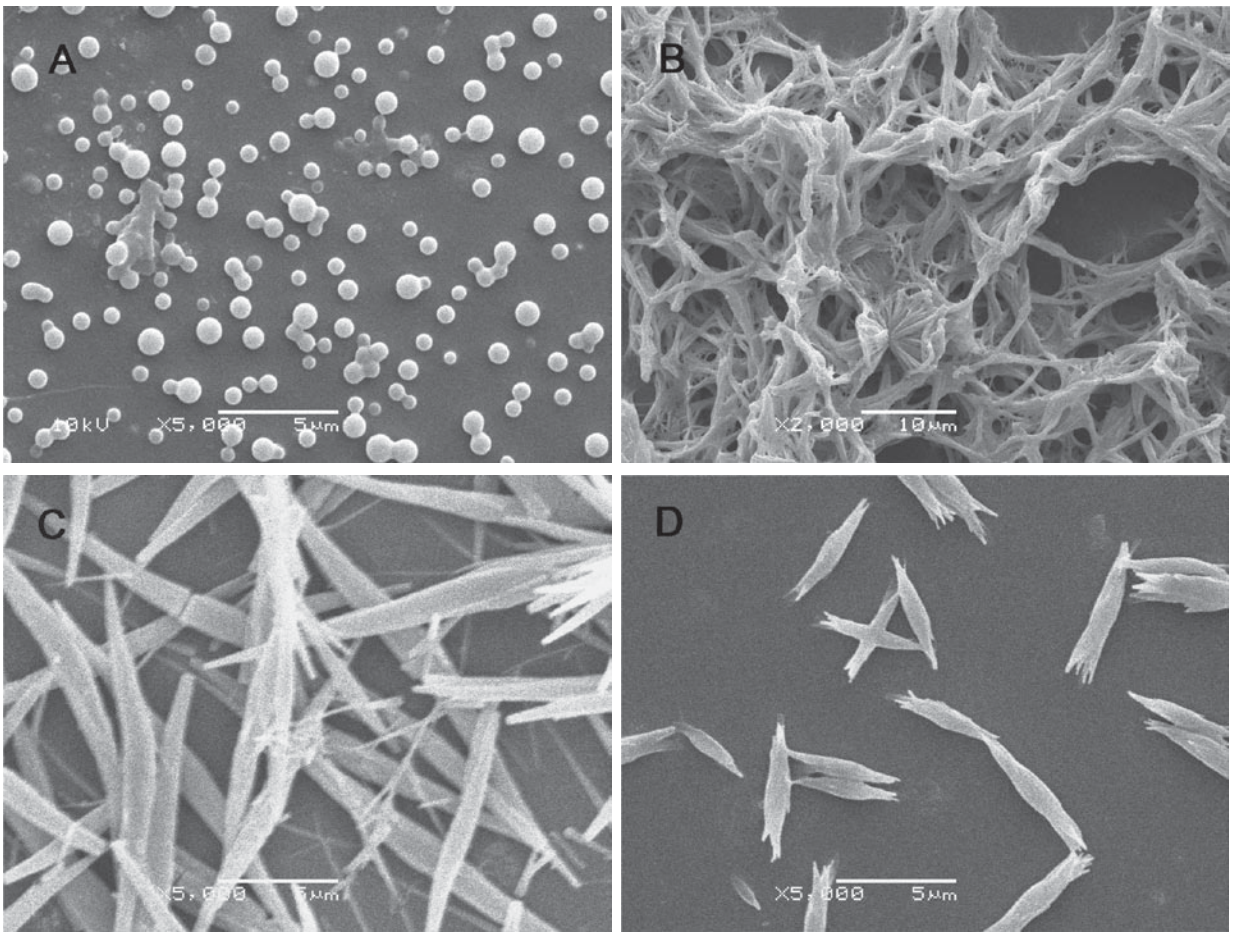

Figure 1. SEM images of (A) amorphous IBS particles, and crystals formed (B) no additive, (C) in the presence of F127, (D) in the presence of T80.

them were effective in stabilizing amorphous drug particles, and the crystallization process was inhibited. In comparison, a high degree of crystallinity was achieved in 10 min when F127 or T80 was used as the additives (Figure 2). As shown in Figure 1C and 1D (10 min), the crystalline particles obtained with F127 and T80 exhibited needle-like shape. For T80, the formed crystalline particles had a width-diameter and length-diameter about 0.8 μm and 5 μm, respectively, which was smaller than the crystalline particles obtained with F127. In order to obtain small crystalline particles, T80 was selected as the crystallization agent to convert amorphous IBS drug into crystalline particles.

To further understand the effect of T80, the crystallization time was investigated in detail. The relationship between the particle size (length-diameter) of crystalline particles and crystallization time is revealed in Figure 3. It could be found that the particle size does not change obviously within 1 h. However, when the crystallization time increases to 2 and 3 h, the particle size increased to 6.5 and 7.6 μm, respectively. Further increasing the crystallization time to 6 h, the particle size dramatically increased to 10 μm. Therefore, it can be concluded that the shorter crystallization time is favorable during the crystallization process. The increasing of particle size with crystallization time can be ascribed to the particle growth during the crystallization process. Therefore, 10 min is believed as the optimum crystallization time to obtain small particles.

The above results indicate that the additives play a key role in accelerating the crystallization rate and controlling particle size of drug particles. Without the additives, although the amorphous drug could be converted into crystalline particles, the crystallization is time-consuming. Moreover, it is an uncontrollable process, and producing larger particles.

Different additives show different influences on the polymorphic form of IBS. PVP or HPMC was not efficient in converting amorphous drug to crystalline particles. This may be due to PVP and HPMC containing hydrophobic chains which can adsorb on the surface of the amorphous drug particles[4,21], and consequently, the particle surface was coated by PVP or HPMC, inhibiting crystallization. In comparison, F127 and T80 were effective in converting the amorphous drug to crystalline particles, which may be attributed to their effect on solubilization (the solubility of IBS: 0.065 mg/mL in water, 0.084 mg/mL in 0.01 wt% F127 solution, 0.075 mg/mL in 0.01 wt% T80 solution, 298 K), where the addition of F127 or T80 can dissolve the surface of the amorphous drug particles and reduce the surface energy. This may reduce the energy of crystallization and promote the crystallization rate[22,23]. T80 was also effective in preventing the growth and aggregation of drug particles, and crystalline particles obtained with T80 were smaller than that of F127. Such an observation may be ascribed to the viscosity of T80 which is higher than that of F127. This may help in decreasing the interfacial tension of the suspension and hence decreasing the particles size[24].

Effect of temperature

Temperature is an important factor influencing the crystallization rate and particles size of IBS. According to the William-Landel-Ferry equation[25], the crystallization rate was determined by increasing the difference between process temperature and glass-transition temperature (T_g). Raising the operating temperature could also accelerate the crystallization rate. However, the higher temperature would lead to the growth of particles. Otherwise, if the operating temperature was too low, the crystallization rate was also too slow. Figure 4 shows the SEM images of IBS particles obtained using T80 as the additive at different operating temperature (crystallization time was 10 min). The particles prepared at 15°C and 25°C were discrete and had the same particle size. However, when the temperature was increased to 40°C, the drug was became lager crystalline particles. Therefore, it can be concluded that when

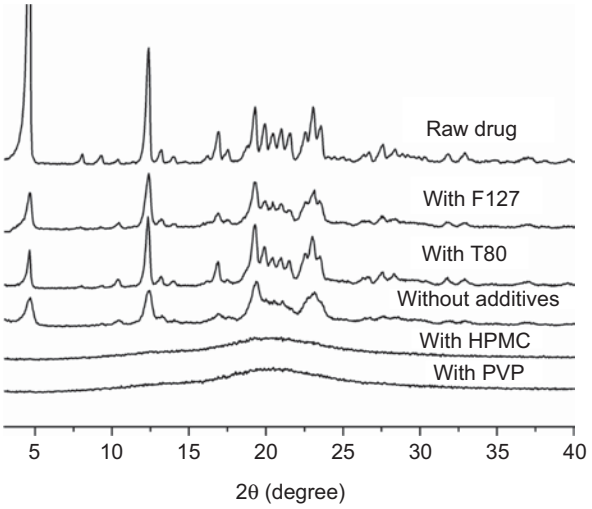

Figure 2. Effect of additives on crystalline polymorphs of IBS (crystallization time: 6 h).

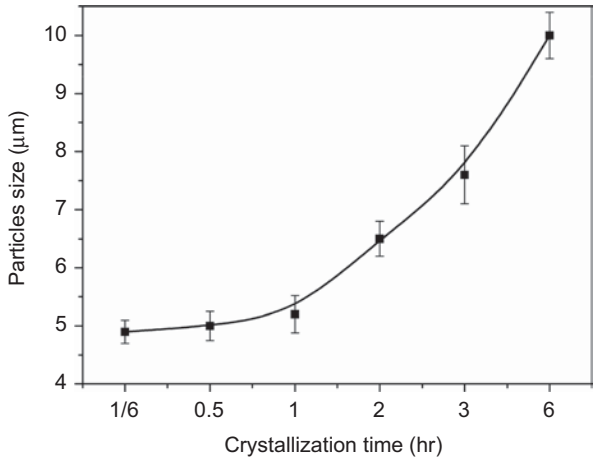

Figure 3. Particles size (length-diameter) of crystalline particles versus crystallization time ($n=3$).

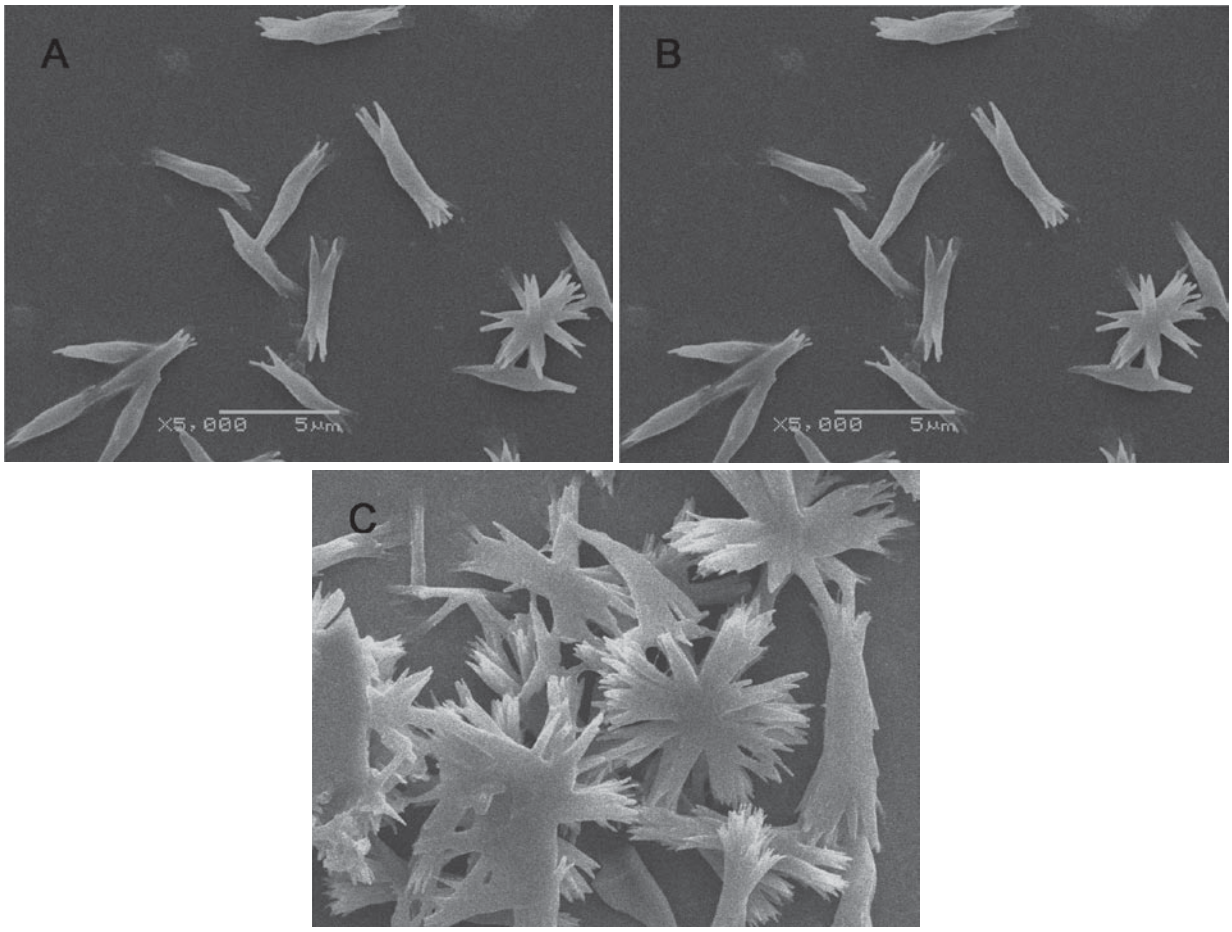

Figure 4. SEM images of IBS particles at different temperature: (A) 15°C, (B) 25°C, (C) 40°C; (crystallization agent: T80, crystallization time: 10 min). All the images share the same scale bar.

the operating temperature was between 15°C and 25°C, the crystallization rate and the growth of particles were balanced.

HPH process

In this study, HPH was employed to further reduce the particle size of crystalline particles. Usually, under certain homogenization cycles, the higher the operating pressure, the smaller the particle size. In the present study, the operating pressure was fixed at 1200 bar. Figure 5 revealed the relationship between homogenization cycles and particle size. It is evident that there is a notable decrease in particle size during the HPH process. When the cycles are 5, the average particle size is about 3 μm. Increasing the cycles to 15, the particle size is decreased to 0.83 μm. However, with the cycles increased to 18 and 20, the average particle size decreased slightly (0.80 and 0.79 μm, respectively). It is worth noting that when the cycles were changed from 15 to 18, the span of particle size distribution (PSD) became narrower. Further increase the cycles to 20, the span was only slightly affected. Therefore, the optimum HPH conditions were 18 cycles under 1200 bar. Figure 6 presents the crystalline particles obtained at the optimum HPH conditions, and the average particle size is about 0.8 μm.

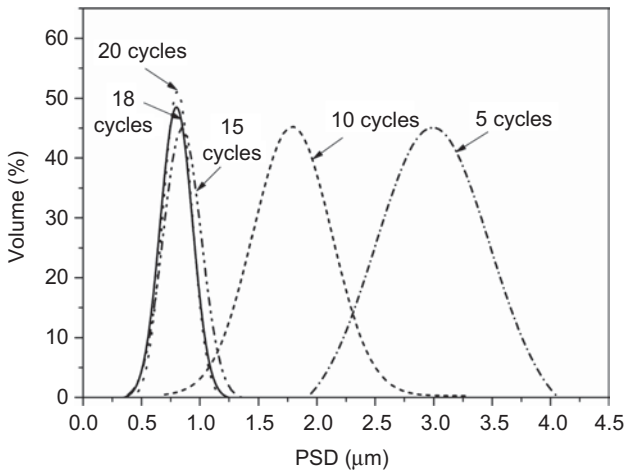

Figure 5. Particles size distribution of crystalline particles vs. homogenization cycles (homogenization pressure: 1200 bar).

Characterization of micron-sized particles and its stability

Freeze-drying is one of the most commonly used methods to convert drug solutions or suspensions into powders of sufficient stability for distribution and storage in the pharmaceutical field[26]. When the crystalline particles were freeze-dried, the obtained powder had good

dispersibility and narrow PSD of 1–4 μm (Figure 7A). After 6 months storage, the morphology and PSD manifested no change.

FT-IR spectroscopy, XRD patterns and DSC curves of raw IBS, fresh micron-sized crystalline particles and micron-sized crystalline particles after storage for 6 months are illustrated in Figure 8. As shown in Figure 8A, it could be seen that the FT-IR spectrum of the fresh micron-sized crystalline particles showed no difference. After storage for 6 months, the spectrum of micron-sized crystalline particles matched well with the raw IBS and fresh micron-sized particles.

The crystalline form of the raw IBS and micron-sized particles were characterized by XRD (Figure 8B). Raw IBS of crystalline form A has peaks expressed as 2θ at 4.8, 12.4, 13.2, 16.9, 19.2, 20.4, 20.9, 21.6, 22.7, 23.2 and 23.4°. The micron-sized crystalline particles had almost the same crystalline peak positions as that of raw IBS between 3° and 40°, indicating that the micron-sized drug was form A, and spectrum showed no obvious change after 6 months. The peaks of the micron-sized particles were lower than those of raw IBS suggesting the smaller size of the micron-sized drug. The XRD results were further confirmed by DSC analysis (Figure 8C). The raw IBS showed a sharp endotherm at 188.9°C (with an enthalpy of 97.3 J/g) corresponding to its melting point, confirming that the raw IBS consisted solely of form A. In comparison, the fresh micron-sized crystalline particles had an endotherm at 185.6°C (with the enthalpy of 92.2 J/g). This reduction in melting point and enthalpy of the micron-sized crystalline particles could be attributed to the decreased particle size, which agreed with XRD analysis. After 6 months, the endotherm of the micron-sized particles appeared at 185.9°C (with the enthalpy of 93.8 J/g). The above results indicate that the micron-sized crystalline particles have good stability.

In vitro dissolution test

In vitro dissolution profiles of the raw IBS, fresh micron-sized crystalline particles and micron-sized crystalline particles after storage for 6 months were compared in Figure 9. The fresh micron-sized crystalline particles reached 60% drug dissolution within 20 min, whereas only 8% of the raw IBS dissolved during the same period. After 120 min, about 80% of the micron-sized crystalline particles were released. However, there was only 26% of raw IBS was dissolved at the end 120 min. The increase of the dissolution rate of the micron-sized crystalline particles is mainly attributed to the reduction of the particle size and the corresponding increase of the specific surface area (from 3.5 m^2/g of raw IBS to 6.9 m^2/g of micron-sized crystalline particles). A minor decrease in the dissolution rate was observed for the micron-sized particles after storage (specific surface area: 6.5 m^2/g). The difference of dissolution rate between the fresh sample and storaged sample was less than 5%. The above results further confirmed that the prepared micron-sized particles were stable.

Conclusions

Micron-sized crystalline IBS particles were successfully prepared to enhance its stability and dissolution rate.

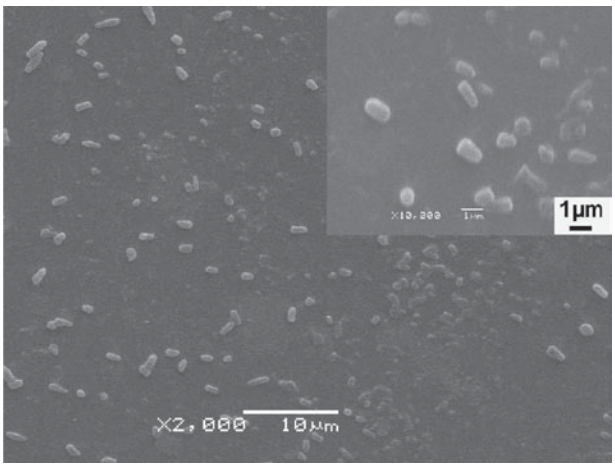

Figure 6. SEM image of homogenized crystalline particles (1200 bar, 18 cycles).

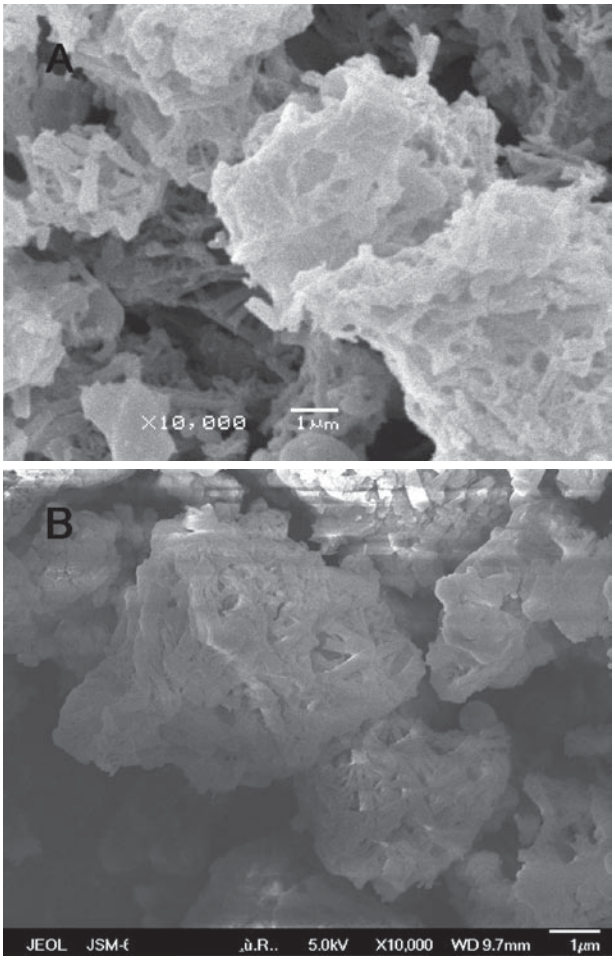

Figure 7. SEM images of IBS powder obtained from freeze-drying (A) fresh sample, (B) after 6 months.

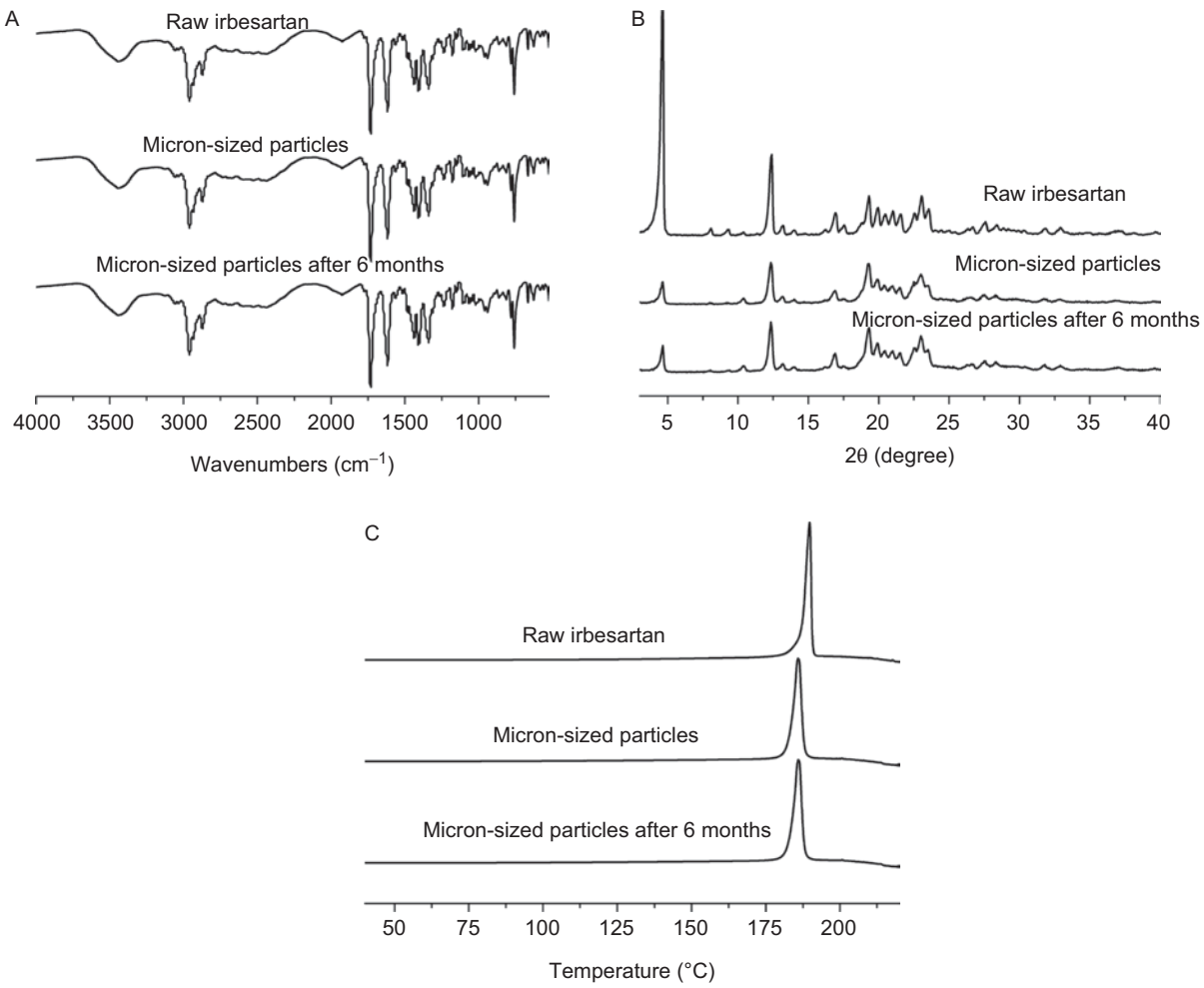

Figure 8. (A) FT-IR spectra, (B) XRD patterns, and (C) DSC curves of raw irbesartan, fresh micron-sized crystalline particles and micron-sized crystalline particles after 6 months.

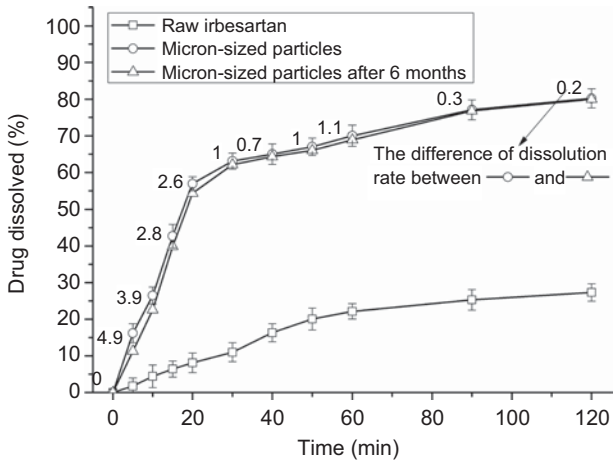

Figure 9. *In vitro* dissolution profile of the raw irbesartan, fresh micron-sized crystalline particles and micron-sized crystalline particles after 6 months ($n=3$, the value upper the curve: the difference of dissolution rate between the fresh micron-sized crystalline particles and micron-sized crystalline particles after 6 months).

Pharmaceutical additives were used as the crystallization agent to convert the liquid precipitated amorphous IBS drug into crystalline particles. The additives tested in this work showed the different effect on the polymorphic form and particle size of IBS. PVP and HPMC were effective in stabilizing amorphous drug particles, and the crystallization process was inhibited. F127 and T80 were effective in converting the amorphous drug to crystalline particles, and T80 was found to be more effective in controlling the particles size than that of F127. After HPH, particles with an average size of 0.8 μm were obtained under 18 cycles at 1200 bar. SEM, FT-IR, XRD, DSC and dissolution rate studies indicated that the freeze-dried micron-sized crystalline particles were stable during 6-month storage. *In vitro* dissolution studies indicated approximately 80% of the micron-sized drug was dissolved after 120 min, while there was only 26% of raw IBS dissolved during the same period. Therefore, preparation of micron-sized crystalline drug particles is an effective way to improve the stability and dissolution rate of IBS.

Declaration of interest

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References

1. Lipinski C. (2002). Poor aqueous solubility, an industry wide problem in drug discovery. Am Pharm Rev, 5:82–85.
2. Rasenack N, Müller BW. (2002). Dissolution rate enhancement by *in situ* micronization of poorly water-soluble drugs. Pharm Res, 19:1894–1900.
3. Müler RH, Peters K. (1998). Nanosuspensions for the formulation of poorly soluble drugs: I. Preparation by a size-reduction technique. Int J Pharm, 160:229–237.
4. Rabinow BE. (2004). Nanosuspensions in drug delivery. Nat Rev Drug Discov, 3:785–796.
5. Matteucci ME, Hotze MA, Johnston KP, Williams RO 3rd. (2006). Drug nanoparticles by antisolvent precipitation: Mixing energy versus surfactant stabilization. Langmuir, 22:8951–8959.
6. Chen X, Matteucci ME, Lo CY, Johnston KP, Williams RO 3rd. (2009). Flocculation of polymer stabilized nanocrystal suspensions to produce redispersible powders. Drug Dev Ind Pharm, 35:283–296.
7. Yassin AE, Alanazi FK, El-Badry M, Alsarra IA, Barakat NS, Alanazi FK. (2009). Preparation and characterization of spironolactone-loaded gelucire microparticles using spray-drying technique. Drug Dev Ind Pharm, 35:297–304.
8. Pouleur HG. (1997). Clinical overview of irbesartan: A new angiotensin II receptor antagonist. Am J Hypertens, 10:318S–324S.
9. Brunner HR. (1997). The new angiotensin II receptor antagonist, irbesartan: pharmacokinetic and pharmacodynamic considerations. Am J Hypertens, 10:311S–317S.
10. Chawla G, Bansal AK. (2008). Improved dissolution of a poorly water soluble drug in solid dispersions with polymeric and non-polymeric hydrophilic additives. Acta Pharm, 58:257–274.
11. Wang Z, Huang Q, Wang J, Shao L, Chen J. (2008). Preparation of amorphous micronized irbesartan by an antisolvent precipitation method. J Beijing Univ Chem Tech, 35:69–73.
12. Hirlekar R, Kadam V. (2009). Preformulation study of the inclusion complex irbesartan-β-cyclodextrin. AAPS Pharmscitech, 10:276–281.
13. Hancock BC, Parks M. (2000). What is the true solubility advantage for amorphous pharmaceuticals? Pharm Res, 17:397–404.
14. Pan X, Julian T, Augsburger L. (2008). Increasing the dissolution rate of a low-solubility drug through a crystalline-amorphous transition: A case study with indomethacin [correction of indomethicin]. Drug Dev Ind Pharm, 34:221–231.
15. de Waard H, Hinrichs WL, Frijlink HW. (2008). A novel bottom-up process to produce drug nanocrystals: Controlled crystallization during freeze-drying. J Control Release, 128:179–183.
16. Hancock BC, Zografi G. (1994). The relationship between the glass transition temperature and the water content of amorphous pharmaceutical solids. Pharm Res, 11:471–477.
17. Chan HK, Chew NY. (2003). Novel alternative methods for the delivery of drugs for the treatment of asthma. Adv Drug Deliv Rev, 55:793–805.
18. Caron A, Chantreux D, Bouloumie C. (1997). Process for the preparation of a tetrazole derivative in two crystalline forms and novel the crystalline forms thereof. US patent no. 5629331.
19. Reddy RB, Sudhakar S. (2003). Amorphous form of 2-n-butyl-3-((2-(1h-tetrazol-5-yl) (1,1′-biphenyl)-4-yl) methyl)-1,3-diazaspiro (4,4′) non-1-en-4-one. World patent no. 03050110.
20. Parthasaradhi RB, Rathnakar RK, Raji RR, Ramakrishna RM. (2004). A novel crystalline form of irbesartan. World patent no. 2004089938.
21. Ziller KH, Rupprecht HH. (1990). Control of crystal growth in drug suspensions. Part II: Influence of polymers on dissolution and crystallization during temperature cycling. Pharm Ind, 52:1017–1022.
22. Muhammad SA, Langrish T, Tang P, Adi H, Chan HK, Kazarian SG et al. (2010). A novel method for the production of crystalline micronised particles. Int J Pharm, 388:114–122.
23. Lu J, Wang X, Ching C. (2003). Effect of additives on the crystallization of lysozyme and chymotrypsinogen A. Crystal Growth & Design, 3:83–87.
24. Zhang ZB, Shen ZG, Wang JX, Zhang HX, Zhao H, Chen JF et al. (2009). Micronization of silybin by the emulsion solvent diffusion method. Int J Pharm, 376:116–122.
25. Williams ML, Landel RF, Ferry JD. (1955). The temperature dependence of relaxation mechanisms in amorphous polymers and other glass-forming liquids. J Am Chem Soc, 77:3701–3707.
26. Lang R, Winter G, Vogt L, Zurcher A, Dorigo B, Schimmele B. (2009). Rational design of a stable, freeze-dried virus-like particle-based vaccine formulation. Drug Dev Ind Pharm, 35:83–97.