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Potent dried drug nanosuspensions for oral bioavailability enhancement of poorly soluble drugs with pH-dependent solubility

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ABSTRACT

The objective of this study was to enhance the oral bioavailability of itraconazole (ITZ) with dried drug nanosuspensions. The feasibility of using poloxamer 407 or HPMC (50 cp) as stabilizers for preparing ITZ nanosuspensions by facile acid–base neutralization was investigated. Dried ITZ nanosuspensions were prepared by spray drying. The effect of matrix former on the dissolution rate of dried ITZ nanosuspensions was investigated. Results from dissolution test revealed that spray-dried ITZ nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2, w/w) preserved the high dissolution rate from nanosuspensions. After oral administration in rats, the AUC_{0-36} from dried ITZ nanosuspensions was 1.5-fold and 1.8-fold higher than the AUC_{0-36} from sporanox pellets (commercial product) in the fed and fasted states, respectively ($p < 0.05$). More importantly, the AUC_{0-36} from dried ITZ nanosuspensions showed no difference between fed/fasted states, because this formulation could enhance the adsorption of ITZ in target site (small intestine) regardless of food intake. In addition, dried ITZ nanosuspensions showed a lower inter-individual variability in terms of bioavailability. Positive results demonstrate that dried drug nanosuspensions formulation prepared by acid–base neutralization combined with spray drying may be a promising method for enhancing the oral bioavailability of poorly soluble drugs with pH-dependent solubility.

1. Introduction

A large number of drugs used today suffer from poor oral bioavailability due to poor aqueous solubility and/or low dissolution rate (Chen et al., 2005; Zimmermann et al., 2009). Developing novel strategies to enhance the solubility of poorly water-soluble drugs is one of the main focuses of pharmaceutical technology. Nanosuspensions are a promising strategy for improving the dissolution rate and oral bioavailability of poorly water soluble drugs by reducing the particle size and/or transforming drugs from a crystalline to an amorphous state. In addition, there is generally a reduction in the variability of fed-fasted state bioavailability with nanosuspensions formulation (Rabinow, 2004; Kesisoglou et al., 2007). Recently, increasing attention has focused on nanosuspensions as witnessed by the rapid increase in the number of reports on this particular area (Chen et al., 2008; Lai et al., 2009; Fakes et al., 2009; Cerdeira et al., 2010; Pardeike and Müller, 2010; Shegokar and Müller, 2010).

There are two general approaches to prepare drug nanosuspensions: precipitation method (bottom-up) and disintegration method (top-down) (Rabinow, 2004). We have reported the feasibility of utilizing an acid–base neutralization reaction (precipitation method) to prepare nanosuspensions for poorly soluble drugs with pH-dependent solubility (Chen et al., 2008). Utilizing acid–base neutralization to prepare nanosuspensions is of particular interest for poorly soluble drugs with pH-dependent solubility because special manufacturing equipment and toxic organic solvents are avoided. It is worth noting that many poorly water soluble drugs exhibit pH-dependent solubility (e.g. itraconazole and amiodarone) (Ruell et al., 2004; Bergström et al., 2004; Kranz et al., 2005). Therefore, utilizing acid–base neutralization to prepare nanosuspensions has considerable potential in the pharmaceutical industry (Chen et al., 2004, 2008).

Itraconazole (ITZ) is a widely used drug for the therapy of both superficial and systemic fungal infections. It is poorly soluble in aqueous media ($S \sim 1$ ng/ml at neutral pH) and exhibits pH-dependent solubility ($S \sim 6$ μg/ml at pH 1) (Ye et al., 2007). In previous studies, various methods including solid dispersion (Kapsi and Ayres, 2001; DiNunzio et al., 2008; Mellaerts et al., 2008), nanosuspensions (Chen et al., 2008), complexation with hydroxypropyl-beta-cyclodextrin (Peeters et al., 2002) and self-microemulsifying formulation (Woo et al., 2008) have been employed to enhance the dissolution rate and/or oral bioavailability of ITZ. As a weak base ($pK_a = 3.7$) (Kapsi and Ayres, 2001), ITZ is soluble in hydrochloric acid solution. Therefore, ITZ is a suitable drug for preparing nanosuspensions by utilizing acid–base neutralization.

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However, nanosuspensions are thermodynamic unstable and often associated with stability issues including Ostwald ripening, aggregation and crystalline transformation (Van Eerdenbrugh et al., 2008). Dried drug nanocrystals are more desirable than nanosuspensions due to their stability and convenience (Kesisoglou et al., 2007; Shegokar and Müller, 2010). However, the drying process is often accompanied by the irreversible aggregation of the nanoparticles, which then decreases the dissolution rate of drugs (Van Eerdenbrugh et al., 2008). Therefore, developing dried drug nanosuspensions that preserve the high dissolution rate for the enhancement of oral bioavailability of poorly water soluble drugs is an important research goal.

In this study, we aimed to enhance the oral bioavailability of ITZ by employing dried ITZ nanosuspensions. The feasibility of using poloxamer 407 or HPMC (50 cp) as stabilizers for preparing ITZ nanosuspensions by facile acid–base neutralization was investigated. The new dried ITZ nanosuspensions formulation (consisting of ITZ, HPMC and mannitol) was prepared by spray drying and the reconstitution properties of dried ITZ nanosuspensions were investigated. Physiochemical characteristics of dried ITZ nanosuspensions were determined by SEM, XRD and X-ray photoelectron spectroscopy (XPS). The effect of mannitol (matrix former) concentration on the dissolution rate of dried ITZ nanosuspensions was also investigated. Furthermore, the oral bioavailability of dried ITZ nanosuspensions was evaluated in rats in the fed and fasted states, which was compared with that of sporanox pellets (the commercial product).

2. Materials and methods

2.1. Materials

ITZ was purchased from Shouguang Fukang Pharmaceutical Co., Ltd. (Shandong, China). Hydroxypropyl methyl cellulose 2910 (HPMC, viscosity of 50 cp) was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Sporanox pellets (containing ITZ, HPMC, starch, PEG, etc.; the size of pellets was approximately 1 mm) were provided by Xian-Janssen Pharm. Ltd. (Xi'an, China). Poloxamer 407 was provided by BASF (Ludwigshafen, Germany). Mannitol was purchased from Sigma (St. Louis, USA). Other chemicals were of HPLC or analytical grade.

2.2. Preparation of ITZ nanosuspensions

ITZ (100 mg) was dissolved in a 1.1 ml mixture of hydrochloric acid solution (3.0 mol/l) and ethanol (1:10, v/v). Stabilizers (50 mg) were dissolved in 10 ml sodium hydroxide solution (containing 15.6 mg sodium hydroxide). This acid solution with ITZ was added to the sodium hydroxide solution at a flow rate of 10 ml/min under moderate stirring, thereby resulting in the desired nanosuspensions. The pH value of the neutralized solution was approximately 7.9 (Chen et al., 2008).

2.3. The measurement of particle size

The average size of nanoparticles in suspension was measured by photon correlation spectroscopy with a Nano ZS90 (Malvern Instruments, UK) at a wavelength of 635 nm at 25 °C. Prior to measurement, samples were diluted with distilled water. All measurements were repeated three times and the average value was used.

2.4. Preparation of dried ITZ nanosuspensions

200% (relative to ITZ weight, w/w) mannitol was added to nanosuspensions prior to spray drying and the mixture was stirred for 5 min. The obtained nanosuspensions was spray dried using a Buchi Mini Spray Dryer B290 (Flawil, Switzerland) with the following parameters: the inlet temperature was 150 °C, outlet temperature was 75 °C, the aspirator air flow was set at 100%, the feeding rate of nanosuspensions was 5 ml/min. ITZ Nanosuspensions was continuously stirred with a magnetic stirrer during the process of spray drying. Dried ITZ nanosuspensions was collected and then stored in sealed bags.

2.5. HPLC analysis

The concentration of ITZ was determined by an appropriate HPLC method (Agilent 1100 series, Agilent, USA). A gemini C_{18} column (250 mm × 4.6 mm 5 μm, Phenomenex Inc., USA) was used for chromatographic separation. The mobile phase consisted of acetonitrile, water and phosphoric acid (63:37:0.026, v/v, pH 2.5). The flow rate was set at 1 ml/min. The UV detector was set at 261 nm. The limit of detection was 0.3 μg/ml. The injection volume was 20 μl. The assay was linear (r^2 = 0.9997) in the concentration range of 1.3–130.0 μg/ml. Approximately 20 mg of spray-dried ITZ nanosuspensions was accurately weighted, which was added to a 100 ml volumetric flask containing 60 ml methanol and then sonicated for 15 min. Afterwards, a volume of 100 ml was attained by adding methanol to the flask. The solution in the flask was filtered through 0.22 μm membrane and then examined by HPLC as mentioned above.

2.6. Reconstitution of dried ITZ nanosuspensions

20 mg of dried ITZ nanosuspensions (ITZ:HPMC:mannitol, 1:0.5:2, w/w) was dispersed in 15 ml of purified water, followed by photographing the process of redispersion.

2.7. Transmission electron microscopy (TEM)

Nanosuspensions were observed using transmission electronic microscope (TEM, FEI, Netherlands). After dilution with purified water, samples were placed on a carbon-coated copper grid and air dried. Samples were then stained with 1% phosphotungstic acid before microscopic analysis.

2.8. Scanning electron microscopy (SEM)

The surface morphology of dried ITZ nanosuspensions was observed using a FEI Sirion-200 field emission scanning electron microscope (SEM, FEI, Netherlands). Prior to observation, samples were coated with a layer of gold using a precision etching coating system (PECS, Gatan, USA). Surface morphologies were obtained at 10 kV.

2.9. X-ray powder diffraction (XRD)

The physical state of ITZ in dried nanosuspensions was verified by X-ray powder diffraction (XRD) using an X'Pert PRO diffractometer (PANalytical, Netherlands). A copper radiation source was used as the anode material. The diffraction pattern was performed in a step scan model with a voltage of 40 kV and a current of 40 mA in the range of $10^\circ < 2\theta < 80^\circ$, with a step size of 0.02°.

2.10. X-ray photoelectron spectroscopy (XPS)

Surface composition was analyzed using X-ray photoelectron spectroscopy (XPS, ThermoVG Scientific, UK) with an analysis depth of less than 10 nm to approximate the distribution of ITZ in dried nanosuspensions. The area of analysis was 8 mm^2.

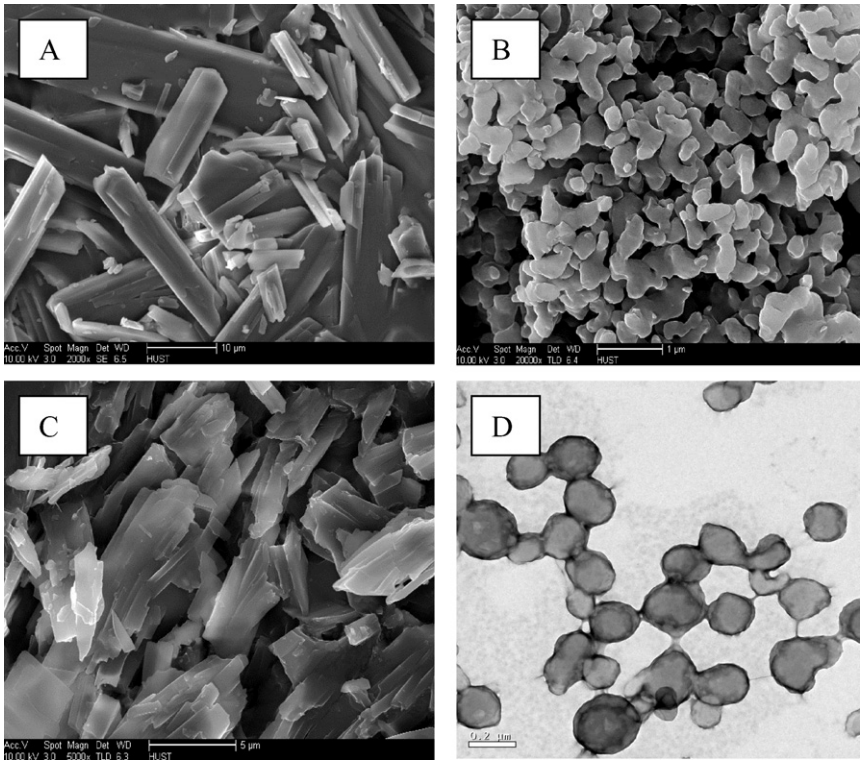

Fig. 1. SEM images of (A) raw ITZ and ITZ particles at 3 h after preparation (B) with no stabilizers, (C) with 50% (relative to ITZ weight, w/w) poloxamer 407 as the stabilizer and (D) TEM image of ITZ nanoparticles with 50% HPMC as the stabilizer.

2.11. Dissolution studies

Dissolution experiments were performed in a dissolution testing apparatus (paddle method) following the Chinese Pharmacopoeia dissolution procedure (2005 ED) (Yu et al., 2010). 900 ml 0.045 mol/l hydrochloric acid solution (the pH value was approximately 1.4) was used as the dissolution medium. The amount of sample for each experiment was equivalent to 100 mg of ITZ. Dissolution experiments were performed at 37 ± 0.5°C with a paddle speed of 50 rpm. Aliquots of 5 ml were taken after 5, 10, 20, 30, 45, 60 min and replaced by an equal volume of fresh dissolution media. The solution was then filtered through a 0.22 μm membrane. The first 2 ml was discarded and the remainder was diluted and analyzed by the HPLC method described above. Dissolution studies were performed in triplicate.

2.12. Pharmacokinetic studies

Animal studies were approved by the Ethical Committee of Huazhong University of Science and Technology. Wister rats (270 ± 20 g) were provided by the Laboratory Animal Center, Hubei Academy of Preventive Medicine (Wuhan, China). All animals were housed in standard cages on a 12 h light-dark cycle with free access to food and water. Rats were divided into four groups (six rats in each group) for oral administration of dried ITZ nanosuspensions or sporanox pellets (containing ITZ, HPMC, starch, PEG, etc.) in the fed and fasted states, respectively. After animals were fed a normal diet or fasted for 12 h, samples at a dose of 15 mg/kg were administrated orally. Dried ITZ nanosuspensions were redispersed in 0.5 ml water prior to oral administration and dosed by an oral syringe. Sporanox pellets were administrated by an oral syringe with 0.5 ml purified water. Blood samples of approximately 500 μl were collected from the orbital plexus into 1.5 ml polyethylene tubes at 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36 h. Polyethylene tubes were flushed with 1% (w/w) heparin solution for anticoagulation. Blood samples were centrifuged and the plasmas were transferred to clean 1.5 ml polyethylene tubes. All samples were stored at −20°C until HPLC analysis (Chen et al., 2008). Plasma samples were removed from −20°C storage and allowed to equilibrate to room temperature before HPLC analysis. 200 μl of plasma and 100 μl of methanol solution containing 0.05% (w/v) pyrene as an internal standard were transferred to a 1.5 ml polyethylene tube. Samples were vortexed for 30 s, and then 600 μl of acetonitrile was added. Samples were vortexed for an additional 3 min, followed by centrifugation at 12,000 rpm for 10 min. Supernatant samples were analyzed using an Agilent 1100 HPLC series. The column and mobile phase were identical to those described in the HPLC analysis. Fluorescence measurements were performed at 261 nm excitation and 365 emission wavelengths. The limit of detection was 5.0 ng/ml. The injection volume was 100 μl. The assay was linear (r^2 = 0.992) in the concentration range of 20–1000 ng/ml.

2.13. Statistical analysis

Data are expressed as the mean ± standard deviation (SD). Statistical analyses were conducted by ANOVA. For all data, a p value of 0.05 was used as the criterion to assess statistical significance.

3. Results and discussion

3.1. Studies on the stabilizer for preparing ITZ nanosuspensions

Prior to spray drying, a stable nanosuspension had to be identified. Studies on the choice of the stabilizer for preparing ITZ nanosuspensions were conducted. When there was no stabilizer in the sodium hydroxide solution, compared with raw ITZ, aggregated nanoparticles of reduced size (500–1000 nm) were formed after acid–base neutralization (Fig. 1A and B). This may be due to the following two-step process: (1) the high degree of supersaturation created by acid–base neutralization resulted in the formation

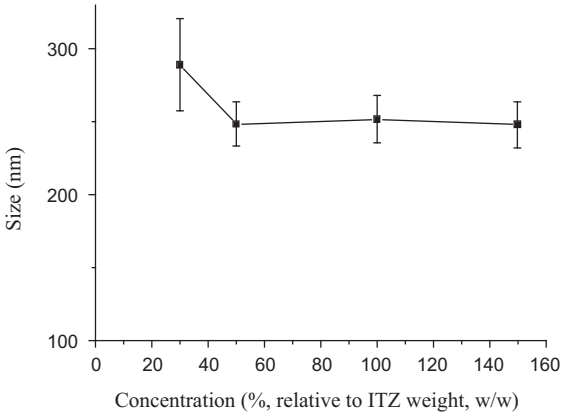

Fig. 2. The influence of HPMC concentrations on average size of ITZ nanoparticles.

of a large number of nanoparticles (Chen et al., 2004), and (2) the nanoparticles aggregated with one another because there was no stabilizer to inhibit aggregation.

The freshly made nanosuspensions had an average size of 256.7 ± 18.3 nm (PDI 0.19 ± 0.06) using 50% (relative to ITZ weight, w/w) poloxamer 407 as the stabilizer, which was not stable. Precipitations were observed at the bottom of nanosuspensions after 30 min. As shown in Fig. 1C, the growth of ITZ particles was enhanced with poloxamer 407 as the stabilizer, compared with the control experiment without stabilizer (in pure water). A similar phenomenon was reported by Zimmermann et al. (2009) when SDS was used as the stabilizer for preparing siramesine microcrystals. It might be explained as follows: (1) there was the affinity interaction between poloxamer 407 and the newly formed ITZ crystal during the process of acid–base neutralization; (2) poloxamer 407 was not effective enough to disperse ITZ nanoparticles and inhibit aggregation of nanoparticles at the high degree supersaturated concentration created by acid–base neutralization; and (3) the poloxamer 407 adsorbed onto ITZ nanoparticles acted as an impurity that enhanced the growth rate of crystals.

As shown in Fig. 1D, spherical ITZ nanoparticles (150–350 nm) were formed when 50% (relative to ITZ weight, w/w) HPMC was used as the stabilizer. In addition, it could be observed that HPMC accumulated on the surface of nanoparticles. During the process of acid–base neutralization, the rapid formation of ITZ nanoparticles increased the interfacial area of the system and drove the amphiphilic HPMC to adsorb onto the surface of nanoparticles, which lowered the interfacial energy. This nanosuspension exhibited good stability over 20 h after preparation. It is probably because HPMC accumulated on the surface of nanoparticles had enough steric hindrance which effectively inhibited the growth and aggregation of ITZ nanoparticles. The stability of ITZ nanosuspensions after 20 h was not investigated because 20 h was a sufficient period of time to spray dry nanosuspensions. Due to the stability concern, HPMC was chosen as the stabilizer for preparing ITZ nanosuspensions. Fig. 2 shows the influence of the concentration of HPMC on the average size of ITZ nanoparticles. The average size of ITZ nanoparticles was decreased from 289.1 ± 31.7 to 248.4 ± 15.1 nm when the concentration of HPMC was increased from 30% (relative to ITZ weight, w/w) to 50%. However, the size of ITZ nanoparticles was not further decreased when the concentration of HPMC was more than 50%. Therefore, 50% HPMC was used as the concentration for preparing ITZ nanosuspensions. Addition of mannitol (matrix former) to the ITZ nanosuspensions did not have the effect on the size of ITZ nanoparticles (Table 1) and the stability of nanosuspensions (data not shown).

Table 1
Average size of nanosuspensions (A ITZ:HPMC 1:0.5, B ITZ:HPMC:mannitol 1:0.5:0.5, C ITZ:HPMC:mannitol 1:0.5:1, D ITZ:HPMC:mannitol 1:0.5:2) which was not dried and nanosuspensions formed by redispersing dried ITZ nanosuspensions (A ITZ:HPMC 1:0.5, B ITZ:HPMC:mannitol 1:0.5:0.5, C ITZ:HPMC:mannitol 1:0.5:1, D ITZ:HPMC:mannitol 1:0.5:2) with 2 min sonication (n = 3).

formulation	Average size (nm)	PDI
ITZ nanosuspensions A	248.4 ± 15.1	0.17 ± 0.06
ITZ nanosuspensions B	248.1 ± 15.9	0.16 ± 0.06
ITZ nanosuspensions C	248.2 ± 16.3	0.17 ± 0.05
ITZ nanosuspensions D	248.5 ± 15.4	0.16 ± 0.07
Redispersed dried ITZ nanosuspensions A	328.9 ± 25.7	0.27 ± 0.14
Redispersed dried ITZ nanosuspensions B	304.2 ± 27.8	0.27 ± 0.17
Redispersed dried ITZ nanosuspensions C	291.7 ± 25.3	0.26 ± 0.15
Redispersed dried ITZ nanosuspensions D	267.3 ± 26.4	0.26 ± 0.13

3.2. Reconstitution properties of dried ITZ nanosuspensions

Spray drying was performed within 20 h after ITZ nanosuspensions preparation. During the process of spray drying, water in nanosuspensions was removed by evaporation, which induced a capillary pressure effect. According to the capillary pressure theory, nanoparticles aggregation was inevitable during the process of spray drying. Aggregations could decrease the dissolution rate of the drug. However, it was possible for dried drug nanosuspensions to maintain the high dissolution rate of nanosuspensions if the aggregation was easy to be redispersed in water in the presence of matrix formers (such as mannitol) (Chaubal and Popescu, 2008). As shown in Fig. 3, dried ITZ nanosuspensions powders with 200% (relative to ITZ weight, w/w) mannitol were found ideally easy to be wetted and redispersed in water. TEM images revealed that dried ITZ nanosuspensions powders with mannitol could be redispersed to nanosuspensions, HPMC accumulated on the surface of nanoparticle could also be observed clearly (Fig. 4). The average size of ITZ nanoparticles in the redispersed dried nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2, w/w) with 2 min of sonication was 267.3 ± 26.4 nm (PDI 0.26 ± 0.13), which was slightly larger compared to the nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2, w/w) (248.5 ± 15.4 nm, PDI 0.16 ± 0.07) that was not dried (Table 1). This may be due to the fact that partial nanoparticles aggregation could not be completely redispersed with 2 min of sonication, which resulted in an increased particle size and PDI. The size and PDI of the redispersed dried ITZ nanosuspensions were even larger than those of ITZ nanosuspensions when the concentration of mannitol was 50% (relative to ITZ weight, w/w) and 100%, respectively

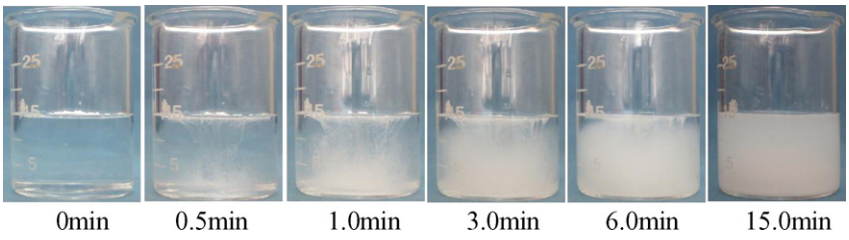

Fig. 3. The redispersion process of 20 mg dried ITZ nanosuspensions (ITZ:HPMC:mannitol, 1:0.5:2, w/w) in 15 ml distilled water.

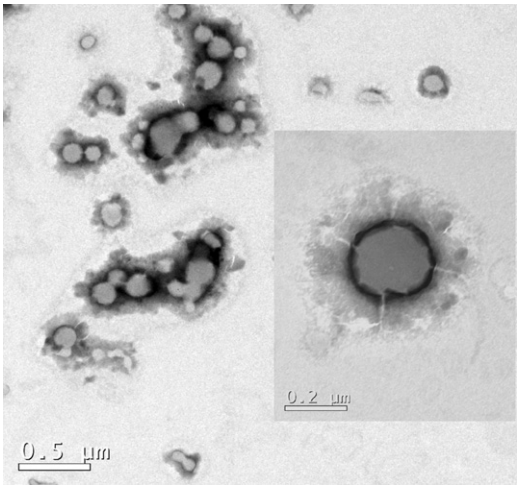

Fig. 4. TEM images of ITZ nanosuspensions formed by redispersing 20 mg dried ITZ nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2) in 15 ml distilled water and incubated at 25 °C for 15 min.

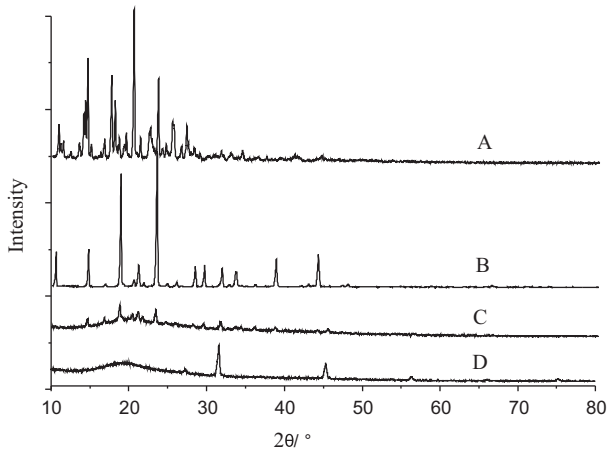

Fig. 6. X-ray powder diffraction patterns of (A) raw ITZ, (B) mannitol, (C) dried ITZ nanosuspensions ITZ:HPMC:manniol 1:0.5:2 and (D) dried ITZ nanosuspensions ITZ:HPMC 1:0.5.

(Table 1). These results imply that these concentrations of mannitol were not enough to redisperse these dried ITZ nanoparticles. Therefore, to make dried ITZ nanosuspensions well redispersed, 200% (relative to ITZ weight, w/w) mannitol was necessary as the matrix former.

3.3. Characterization of dried ITZ nanosuspensions

SEM images of spray dried ITZ nanosuspensions are shown in Fig. 5. When there was no matrix former, spray dried particles were not regular (Fig. 5A). Many ITZ nanoparticles that were approximately 200–500 nm in diameter aggregated tightly in these dried particles (Fig. 5A and B). With 200% (relative to ITZ weight, w/w) mannitol as the matrix former, dried ITZ nanosuspensions consisted of relatively regular particles. This finding suggests that mannitol had the ability to inhibit the aggregation of spray dried particles (Fig. 5C). The numbers of ITZ nanoparticles in a single dried particle with mannitol were reduced, thereby revealing that mannitol could reduce the degree of nanoparticles aggregation in dried particles (Fig. 5D).

To assess the physical state of ITZ in the dried nanosuspensions, XRD was performed. As shown in Fig. 6C, characteristic peaks of crystalline ITZ almost disappeared when dried ITZ nanosuspensions with mannitol was tested; XRD patterns were very similar to that of pure mannitol, which corresponded to polymorphism mannitol (Burger et al., 2000). To eliminate the influence of mannitol, dried ITZ nanosuspensions without mannitol was determined. As shown in Fig. 6D, characteristic peaks of crystalline ITZ completely disappeared, thereby indicating that ITZ existed as an amorphous state in the dried nanosuspensions without mannitol. The diffraction patterns in Fig. 6D exhibited characteristic peaks of sodium chloride

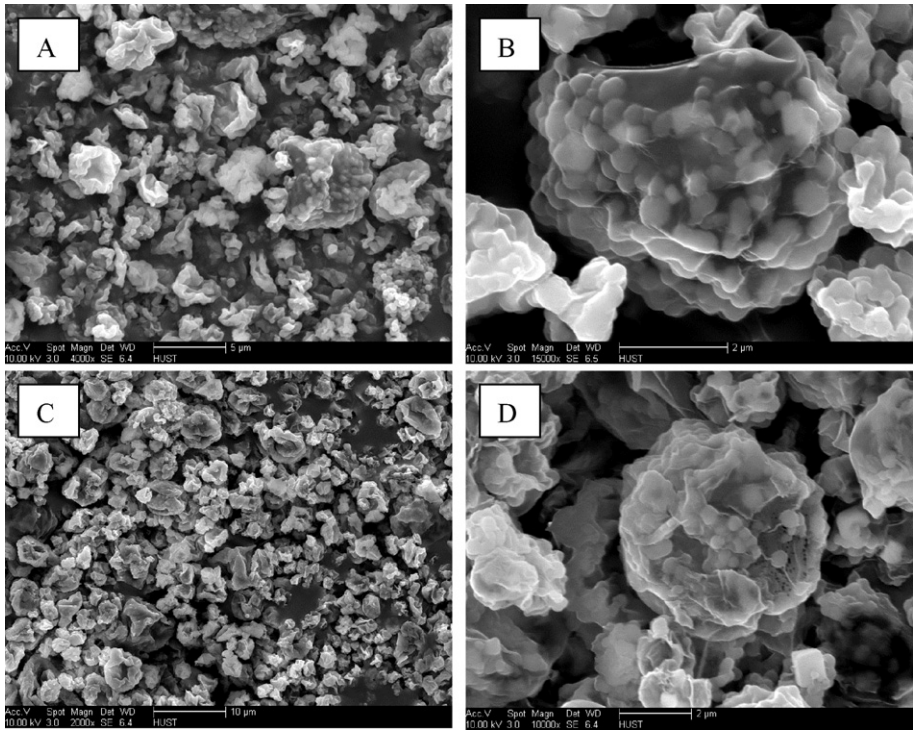

Fig. 5. SEM images of spray-dried ITZ nanosuspensions. (A and B) ITZ:HPMC 1:0.5 and (C and D) ITZ:HPMC:mannitol 1:0.5:2.

Table 2
Nitrogen atoms integrated area of raw ITZ and dried ITZ nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2).

Composition	Nitrogen atoms integrated area	Approximate percentage of ITZ on the surface of dried nanosuspensions (%)
Raw ITZ	12,088	
Dried ITZ nanosuspensions	525	15.2

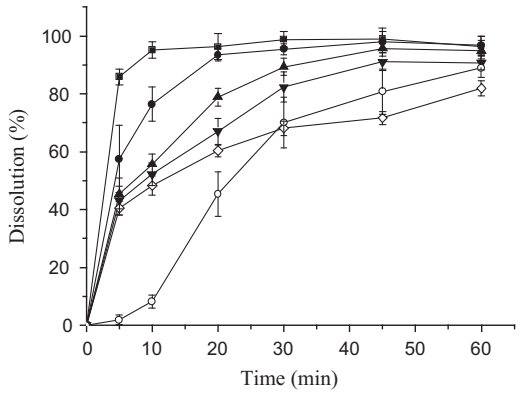

Fig. 7. Dissolution profiles of (■) ITZ nanosuspensions (ITZ:HPMC 1:0.5), (●) dried ITZ nanosuspensions ITZ:HPMC:manniol 1:0.5:2, (▲) dried ITZ nanosuspensions ITZ:HPMC:manniol 1:0.5:1, (▼)dried ITZ nanosuspensions ITZ:HPMC:manniol 1:0.5:0.5 (◊) dried ITZ nanosuspensions ITZ:HPMC 1:0.5 and (○) sporanox pellets in pH 1.4 hydrochloric acid solution, mean ± SD (n = 3).

deriving from the acid–base neutralization reaction (Chen et al., 2008). Therefore, it could be concluded that ITZ was also amorphous state in dried ITZ nanosuspensions with mannitol.

Surface composition analysis was performed using XPS with an analysis depth of less than 10 nm to reveal the distribution of ITZ in the dried nanosuspensions. Because ITZ contains nitrogen atoms, whereas HPMC and mannitol do not have any nitrogen atoms, the distribution of ITZ on the surface of dried nanosuspensions was approximated from the integrated area of the nitrogen curve, as reported elsewhere (Matteucci et al., 2007). For raw ITZ, the integrated nitrogen area was 12,088 while that of the dried nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2) was 525. The predicted integrated nitrogen area was 3453 (12,088/(1 + 0.5 + 2)) for random mixing in the formulation (ITZ:HPMC:mannitol 1:0.5:2). Therefore, ITZ on the surface of dried nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2) was approximately 15.2% (525/3453) (Table 2), thereby indicating good coverage of ITZ nanoparticles by HPMC and mannitol.

3.4. Dissolution studies

The dissolution rate of ITZ from nanosuspensions was approximately 96.3% in the initial 20 min when pH 1.4 hydrochloric acid solution was used as the dissolution medium (Fig. 7). The rapid release of ITZ from nanosuspensions could be attributed to ITZ nanoparticles with a smaller particle size, amorphous state and better hydrophilic property. The dissolution rate of dried ITZ nanosuspensions without the matrix former was only 60.3% in the initial 20 min (Fig. 7). Partial nanoparticles irreversible aggregation (Table 1) and the decreased surface hydrophilicity may have been responsible for the decreased dissolution rate. The dissolution rate of dried ITZ nanosuspensions increased with increasing of mannitol concentration in dried nanosuspensions from 50% to 200% (relative to ITZ weight, w/w). The dissolution rate of dried ITZ nanosuspensions powder with 200% mannitol (ITZ:HPMC:mannitol 1:0.5:2) was approximately 93.5% during the initial 20 min and was 2.1-fold higher than that of sporanox pellets, although a slight decrease was found in comparison with that of the nanosuspensions (Fig. 7). This result revealed that spray dried ITZ nanosuspensions could preserve the high dissolution rate from nanosuspensions in the presence of an appropriate matrix formers. During the process of spray drying, 200% mannitol in nanosuspensions formed enough much water soluble barriers around ITZ nanoparticles. XPS results revealed that ITZ nanoparticles in dried particles were well covered by mannitol and HPMC. According to XPS results and TEM images of the redispersed dried nanosuspensions, it was reasonable to believe that irreversible aggregation of nanoparticles was effectively inhibited by HPMC and mannitol. Many micro-channels were formed when dried ITZ nanosuspensions was exposed to the dissolution media, which was due to the rapid dissolution of mannitol around nanoparticles. The capillary pressure drove much the dissolution media into dried ITZ nanosuspensions powder through these micro-channels and made the powder easier to be redispersed, and then accelerated the dissolution. Effective inhibition of the nanoparticles irreversible aggregation was the key factor for dried ITZ nanosuspensions to maintain the high dissolution rate (Van Eerdenbrugh et al., 2008).

Table 4
The comparison of the pharmacokinetic parameters of dried ITZ nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2) and sporanox pellets in the fed and fasted states.

Parameter	Comparison	Mean ratio
AUC_{0-36}	Fed state: dried ITZ nanosuspensions/sporanox pellets	1.5*
	Fasted state: dried ITZ nanosuspensions/sporanox pellets	1.8*
	Dried ITZ nanosuspensions: fed state/fasted state	1.0
	Sporanox pellets: fed state/fasted state	1.2

* $p < 0.05$.

3.5. Pharmacokinetic studies

To investigate whether dried ITZ nanosuspensions could enhance the oral bioavailability of ITZ, pharmacokinetic studies were performed in rats. Dried ITZ nanosuspensions with 200% mannitol were carried out because it preserved the improved dissolution rate from nanosuspensions. The pharmacokinetic parameters and comparisons are listed in Tables 3 and 4. Curves of the average plasma concentration over time are shown in Fig. 8. After oral administration, the C_{max} of dried ITZ nanosuspensions in the fed and fasted states were 584.3 ± 142.1 ng/ml and 671.2 ± 170.6 ng/ml, respectively, which was higher than those of sporanox pellets (Table 3). Especially in the fasted state, the C_{max}

Table 3
The pharmacokinetic parameters after oral administration of dried ITZ nanosuspensions (ITZ:HPMC:mannitol 1:0.5:2) and sporanox pellets to rats (n = 6).

Parameters	Dried ITZ nanosuspensions		Sporanox pellets	
	Fed state	Fasted state	Fed state	Fasted state
T_{max} (h)	4.9 ± 3.5	4.7 ± 3.1	2.4 ± 0.8	4.7 ± 2.3
C_{max} (ng/ml)	584.3 ± 142.1	671.2 ± 170.6	521.6 ± 93.4	362.5 ± 98.2
AUC_{0-36} (ng h/ml)	12236.8 ± 895.7	12107.8 ± 954.2	7920.3 ± 1426.1	6712.4 ± 1275.1

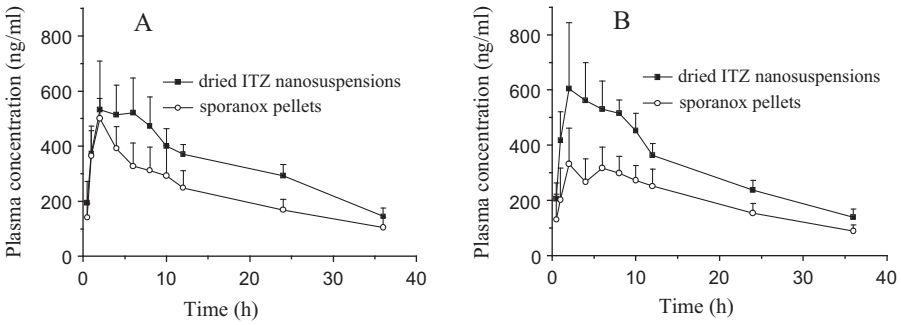

Fig. 8. Mean plasma concentration versus time profiles of ITZ in rats after oral administration of dried ITZ nanosuspensions (ITZ:HPMC:manniol 1:0.5:2) and sporanox pellets at a dose of 15 mg/kg ($n = 6$): (A) in the fed state and (B) in the fasted state.

of dried ITZ nanosuspensions was 1.9-fold greater than that of sporanox pellets (referring to Table 3). In the fasted state, sporanox pellets (containing ITZ, HPMC, starch, PEG, etc.) passed through the empty stomach much faster and weakly basic ITZ in sporanox pellets did not have enough time to be easily dissolved in the acid environment. The dissolution rate of ITZ from dried ITZ nanosuspensions was faster than that from sporanox pellets, which resulted in the greater C_{max}. When ITZ was administrated as dried nanosuspensions formulation, the plasma concentration was maintained at a high level for a long time regardless of food intake, which led to the enhancement of bioavailability (Fig. 8). The AUC_{0-36} of dried ITZ nanosuspensions was 1.5-fold and 1.8-fold higher, respectively than the AUC_{0-36} of sporanox pellets in the fed state and fasted states ($p < 0.05$) (Table 4). More importantly, the AUC_{0-36} from dried ITZ nanosuspensions showed no difference between fed/fasted states, whereas food intake showed a positive effect on the adsorption of sporanox pellets. This result revealed that dried ITZ nanosuspensions could enhance the adsorption of ITZ in target site (small intestine) regardless of food intake. It might be that the dissolution rate of nanosized ITZ formulation was fast enough in vivo even in the fasted state which reduced the dependence of ITZ dissolution on the presence of food.

According to the biopharmaceutical classification system (BCS), ITZ is a BCS II drug, which means that the dissolution rate is the primary limiting step for the adsorption of ITZ (Amidon et al., 1995). The high dissolution rate of ITZ in the small intestine (target site) is necessary for the bioavailability enhancement (Woo et al., 2008). The high AUC_{0-36} values from dried ITZ nanosuspensions implied that good adsorption occurred after the fast dissolution of ITZ from nanoparticles in the gastrointestinal tract. In addition, nanoparticles could stay a longer time in the gastrointestinal tract due to the adhesive property (Jinno et al., 2006; Shegokar and Müller, 2010). Therefore, ITZ could be continuously released from nanoparticles in vivo, which led to larger drug concentrations for adsorption. As a result, the oral bioavailability of ITZ was enhanced. Additionally, dried ITZ nanosuspensions showed a low inter-individual variability in terms of bioavailability, which should be attributed to the enhanced adsorption area and more easy ITZ release from nanosized drug particles.

4. Conclusions

Dried ITZ nanosuspensions were prepared by acid–base neutralization combined with spray drying. Physiochemical characteristics of dried ITZ nanosuspension were determined. Reconstitution properties revealed that dried ITZ nanosuspensions with an appropriate matrix former could be easily redispersed to nanosuspensions. Dissolution tests revealed that dried ITZ nanosuspensions with the matrix former preserved the high dissolution rate from nanosuspensions. After oral administration in rats, the AUC_{0-36} from dried ITZ nanosuspensions was significantly enhanced compared to that from sporanox pellets (the commercial product) in the fed and fasted states, respectively ($p < 0.05$). In addition, dried ITZ nanosuspensions showed no difference in adsorption between fed/fasted states and a low inter-individual variability in bioavailability. Positive results demonstrate that dried drug nanosuspensions formulation prepared by acid–base neutralization combined with spray drying may be a promising method for the enhancement of oral bioavailability of poorly soluble drugs with pH-dependent solubility.

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