European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 426–432

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Novel Tanshinone II A ternary solid dispersion pellets prepared by a single-step technique: In vitro and in vivo evaluation

Jin Li, Pan Liu, Jian-Ping Liu *, Wen-Li Zhang, Ji-Kun Yang, Yong-Qing Fan

Department of Pharmaceutics, China Pharmaceutical University, Nanjing, PR China

ARTICLE INFO

Article history:
Received 12 May 2011
Accepted in revised form 7 November 2011
Available online 18 November 2011

Keywords:
Ternary solid dispersion pellets
Combined carriers
Polyvinylpyrrolidone
Poloxamer 188
In vitro dissolution study
In vivo evaluation

ABSTRACT

Novel Tanshinone II A (TA) ternary solid dispersion (tSD) pellets with the combination of polyvinylpyrrolidone and poloxamer 188 as dispersing carriers were prepared by a single-step technique. A formulation screening study showed that the addition of poloxamer 188 to binary TA-PVP system could remarkably promote the dissolution rate of TA from 60% to 100% after 60 min. Scanning electron microscopy study revealed a smooth surface and a tightly packed coating structure. Differential scanning calorimetry analysis confirmed the formation of solid dispersions. In vivo test showed that TA tSD pellets presented significantly larger AUC_{0-t}, which was 0.76 times more than that of binary solid dispersion (bSD) pellets, 2.87 times more than that of physical mixtures (PMs) and 5.40 times more than that of TA. C_{max} of TA tSD pellets also increased by 1.82–8.97-fold as that of bSD pellets, PMs and TA. TA tSD pellets generated obviously shortened T_{max} of (3.80 ± 0.398) h, compared to bSD pellets with (4.15 ± 0.456) h, PMs with (4.65 ± 0.226) h and TA with (5.52 ± 0.738) h. In conclusion, the addition of poloxamer 188 to pellets containing PVP-based solid dispersions could achieve complete dissolution, accelerated absorption rate and superior oral bioavailability. The fluid-bed technique becomes an alternative approach to obtain solid dispersion-coated pellets.

1. Introduction

Tanshinone IIA (TA), one of the liposoluble bioactive constituents extracted from the root of Salvia miltiorrhiza Bunge, exhibits a variety of cardiovascular activities including vasorelaxation and a cardio-protective effect [1–3].

However, TA has a low oral bioavailability [4] due to its negligible solubility in water (2.8 ng/mL) [5], insufficient dissolution rate [6,7] and first pass metabolism [8]. Currently, numerous pharmaceutical strategies have been employed to overcome the poor dissolution rate of TA, e.g., TA inclusion compound with HP-β-CD [6], TA dried emulsions, TA solid dispersions [9] and so on. Nevertheless, the resultant intermediate products still needed to be formulated into dosage forms before application.

Pellets, as a multiple unit dosage form, possess many advantages over conventional solid dosage forms, such as flexibility of drug release adjustment by coating or unit combination, reducing intrasubject and intersubject variability of plasma profiles and minimizing GI irritation without lowering drug bioavailability [10]. However, for poorly water-soluble drugs, e.g., TA, pellets cannot satisfactorily solve the problems of absorption and bioavailability.

Solid dispersions (SDs) have been widely applied to enhance the dissolution rate and oral bioavailability of poorly water-soluble drugs [11–14], among them binary solid dispersions (bSDs) composed of a hydrophilic carrier in which the drug is dispersed were extensively described in literature. However, recent studies have shown that, during the accelerated dissolution of bSDs, an unavoidable supersaturation of drug and the consequent recrystallization [15–18], especially when there is a large solubility difference between carrier and drug [17,19], may lead to decreased solubility and dissolution rate. Surfactants were incorporated into the binary drug-polymer systems to constitute the ternary solid dispersion systems [18,20–22], which could avoid undesired drug recrystallization and improve drug wettability, thus potentiate the delayed dissolution.

Researchers have made numerous attempts to obtain solid dispersions in a final pellet form. Jachowicz et al. [23], for example, prepared ketoprofen solid dispersion pellets by a dissolution–evaporation process and subsequent extrusion and spheronization. However, there is the possibility that during extrusion–spheronization process, the mechanical stress, moisture and other factors may increase drug migration and promote drug crystallization in solid dispersions [24], resulting in poor stability, decreased solubility and dissolution rate. In previous studies, a single-step technique of deposition of solid dispersion solutions onto the surface of pellet was reported to form SDs-coated pellets [25,26]. This

* Corresponding author. Department of Pharmaceutics, China Pharmaceutical University, No. 24 Tong jia xiang, Nanjing, PR China. Tel./fax: +86 25 83271293.
E-mail address: liujianpingljp@hotmail.com (J.-P. Liu).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.11.003

was a simplified manufacturing process, which would be more suitable for continuous operation in large-scale production. Therefore, the same technology was applied to minimize complexities during the preparation of TA ternary solid dispersion pellets (TA tSD pellets).

The present study was to prepare TA tSD pellets composed of a hydrophilic polymer (PVP) and a surfactant (poloxamer 188) as dispersing carries by a single-step fluid coating method. The effect of incorporation of poloxamer 188 on TA dissolution was assessed to screen the optimum formulation. DSC measurement was applied to confirm the absence of crystallinity in tSD pellets. The morphology of pellets was characterized by SEM analysis. Bioavailability of TA tSD pellets in rabbits was investigated as compared to TA, its PMs and bSD pellets.

2. Materials and methods

2.1. Materials

TA (98.63%) was purchased from Xi′an honson biotechnology Co., Ltd. TA standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Sugar spheres (0.75–0.85 mm) were from JRS Pharma (Germany). PVP-K30 was kindly donated by China Division, ISP Chemicals Co. (Shanghai, China). Poloxamer 188 (Pluronic®F68) was obtained from BASF Aktiengesellschaft (Ludwigshafen, Germany). Gelatin capsules (type 0) were obtained from Suzhou Capsugel Ltd. All reagents were of analytical grade except methanol and acetonitrile of chromatographic grade.

2.2. Animals

Healthy male New Zealand rabbits (body weight 2.0 ± 0.9 kg) were purchased from Experimental Animal Center of China Pharmaceutical University (Nanjing, China). Prior to the experiments, the rabbits were housed in a temperature and humidity controlled room (23 °C, 55% air humidity) with free access to water and standard rabbit chow. The rabbits were acclimated for at least 5 days and fasted overnight but supplied with water ad libitum before the experiments. All experiments were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University.

2.3. Preparation of TA tSD pellets

2.3.1. Preparation of sprayed solution

After several trials, it was found that ethyl acetate and anhydrous ethanol at a volume ratio of 5:1 were able to adequately dissolve TA, PVP and poloxamer 188. Two solutions were prepared. In solution A, PVP and poloxamer 188 were dissolved in 40 mL of anhydrous ethanol solution. In solution B, TA was dissolved in ethyl acetate solution with a final concentration of 5 mg/mL. Solution A was added slowly into solution B with adequate stirring to ensure complete mixing of the solutions. Followed by ultrasonication for 10 min using an ultrasound cleaner (DL-720A, Shanghai, China) until a clear solution was obtained.

2.3.2. Deposition of TA tSD on sugar pellets

Deposition of TA/PVP/poloxamer 188 ternary solid dispersions on sugar pellets was performed in a fluid-bed granulator and coater (JHQ-100, Shenyang, China). Firstly, 5 g sugar pellets were fluidized by opening the inlet air flap till the outlet temperature reached 35 °C. The solution was then bottom-sprayed onto sugar pellets from a nozzle (0.5 mm) attached to a peristaltic pump (HL-2, Shanghai, China) under the condition of 100–150 mL min^{-1} air flow rate, 1.0 mL min^{-1} spray rate and 1.5–1.6 bar atomizing air pressure. After drug/carriers layering, the pellets were dried for a further 15 min at 30–35 °C in the coating chamber. All operations were protected from light exposure. The morphology with the naked eye, the coating weight and the recoveries of the resulting pellets were assessed to determine the feasibility in pilot tests.

2.4. In vitro dissolution study

2.4.1. Determination of TA

Concentrations of TA in dissolution medium were quantified by high pressure liquid chromatography (HPLC, Shimadzu LC-20A, Kyoto, Japan) equipped with a diode array detection (DAD) set at 268 nm[27]. The separation was performed at 30 °C on a Synergi Hydro-RP C18 column (5 μm, 250 mm × 4.6 mm, Phenomenex, USA) protected by a C18 Securityguard column (5 μm, 10 mm × 4.6 mm, Kromasil, Sweden). The mobile phase was methanol/water (90:10, v:v) and delivered at a flow rate of 1.0 mL min^{-1}. The injection volume was 20 μL. The linearity of the method was studied in the concentration range of 0.5–5.0 μg mL^{-1} (r = 0.9997). The RSD of the intraday and interday precision for TA were less than 2%. The recovery rates for TA were in the range of 98–102%, and the RSD were below 2%.

2.4.2. In vitro dissolution test

Dissolution experiments were performed in USP 27 XXIII Dissolution Apparatus I (rotating basket method) (ZRS-8G dissolution apparatus, Tianjin, China). Samples containing 1.5 mg of TA were sealed in hard gelatin capsules with a manual capsule filling machine (CapsulCN, Zhejiang, China), then put into the rotating basket and immersed in the dissolution medium (900 mL of distilled water contained 0.5% sodium dodecyl sulfate) thermostatically maintained at 37 ± 0.5 °C at a rotation rate of 100 rpm. At predetermined time intervals, 5 mL of the sample was withdrawn and the same volume of fresh dissolution medium was supplemented after each point to keep constant volume. The samples were filtered through 0.22 μm filter and analyzed by HPLC for TA as described above. The dissolution profiles of formulations with and without poloxamer 188 were compared to confirm the prominent effect of combined carriers. Different ratios of TA, PVP and poloxamer 188 were also investigated to screen the optimal formulation. Table 1 shows the quantitative composition (theoretical) of the formulations (binary/ternary solid dispersion pellets) used in this study. Dissolution experiments were performed in triplicate and the average dissolution profiles, and standard deviations were calculated.

Table 1
Composition (theoretical) of the binary/ternary solid dispersion pellets.

Formulations	Composition	Ratios
PMs	TA/PVP	1/2
	TA/PVP	1/6
	TA/PVP/poloxamer 188	1/4/1
	TA/PVP/poloxamer 188	1/6/1
bSD pellets	TA/PVP	1/1
	TA/PVP	1/2
	TA/PVP	1/4
	TA/PVP	1/6
tSD pellets	TA/PVP/poloxamer 188	1/1/1
	TA/PVP/poloxamer 188	1/2/1
	TA/PVP/poloxamer 188	1/4/0.5
	TA/PVP/poloxamer 188	1/4/1
	TA/PVP/poloxamer 188	1/4/2

2.5. Characterization of TA tSD pellets

2.5.1. Scanning electron microscopy (SEM)

The shape, surface and cross-section morphology of the TA tSD pellets were examined using a scanning electron microscope (S-3000N, Hitachi, Japan). Prior to the examination, samples were fixed on a brass specimen club by double-side adhesive tape and made electrically conductive by coating in a vacuum (6 Pa) with platinum (6 nm/min) using a Hitachi Iron Sputter (E-1030) for 300s at 15 mA.

2.5.2. Differential scanning calorimetry (DSC)

In this paper, sugar pellets were used as a substrate to load SDs rather than an ingredient mixed within the composition of SDs. Thus, the TA/PVP/poloxamer 188 solid dispersion samples were prepared by spraying into the drying chamber without sugar pellets under the same coating conditions, and then the solid dispersions were peeled off carefully and grounded to the fine powder for DSC analysis.

Thermal analysis of the samples (TA ternary solid dispersions, physical mixtures, TA, PVP and poloxamer 188) were carried out with a DSC 204A/G Phoenix® instrument (Netzsch, Germany). About 10 mg of sample was weighed into a non-hermetically sealed aluminum pan. The samples were heated from 25 to 300 °C at a heating rate of 10 °C/min. An empty pan was used as reference. All the DSC measurements were made in a nitrogen atmosphere, and the flow rate was 100 mL min^{-1}.

2.6. In vivo study

2.6.1. Animal experiment

Twenty-four male New Zealand rabbits (body weight 2.0 ± 0.9 kg) divided randomly into four groups were fasted for 12 h, but allowed to take water freely. Four formulations (TA, its bSD pellets with TA-to-PVP ratio of 1:4, its PMs and tSD pellets with TA/PVP/poloxamer 188 ratio of 1:4:1) at a dose equivalent to 30 mg/kg of TA were filled into size 0 hard gelatin capsules with a manual capsule filling machine (CapsulCN, Zhejiang, China) and orally administered to four groups of rabbits ($n = 6$), respectively. Plasma samples (1.5 mL) were collected from auricular vein initially and at 0 (pretreatment), 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h after dosing. Plasma was separated by centrifugation at 3000 rpm for 10 min and stored at −20 °C until analysis.

2.6.2. Plasma sample processing and validity

Prior to extraction, frozen plasma samples were thawed at ambient temperature. Sample preparation was carried out under subdued light. A total of 200 μL of rabbit plasma was pipetted in a 10 mL conical centrifuge tube. A single-step precipitation protein procedure was adopted to extract TA from rabbit plasma. Firstly, 400 μL acetonitrile was added and vortex-mixed for 3 min to fully precipitate protein. Secondly, the mixture was centrifuged at 3000 rpm for 10 min. Then, the supernate was transferred to a clean centrifuge tube and dried under a stream of nitrogen at 40 °C water bath. The residue was resuspended in 200 μL methanol and centrifuged at 12,000 rpm for 10 min. Aliquots (20 μL) of the supernate were injected into the HPLC system for analysis, and the chromatographic condition was consistent with Section 2.4.1.

2.6.3. Data presentation and analysis

Pharmacokinetic parameters, including AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and T_{max}, were calculated by compartmental analysis using the software program PKSolver [28,29]. Data reported were arithmetic mean values ± standard deviation (Mean ± SD). Using two-tailed Student's *t*-tests, *p*-values were calculated between the different pairs of formulations.

3. Results and discussion

3.1. Preparation of TA tSD pellets

Observed by eye, the resultant pellets were intact spherical in shape and had no obvious defects in the coating. At a coating weight gain of about 100%, the diameter of the pellets just increased from 0.75–0.85 mm to about 1 mm, which was beneficial for formulation development. The recoveries of pellets were greater than 90%, indicating that the process is reproducible with an acceptable recovery for pilot test. From a practical point of view, the amount of pellets equivalent to 30 mg of TA, a common clinical dose, could be easily encapsulated into a size 0 hard gelatin capsule, which would facilitate the compliance of patients in medicine taking.

3.2. Formulation investigations into TA tSD pellets

3.2.1. Effects of TA/PVP ratio on dissolution of TA

Solid dispersions are usually two-component systems consisting of drug and a hydrophilic carrier [30]. PVP is a commonly used water-soluble carrier due to its good solubility in water and several organic solvents and its ability to inhibit recrystallization of dispersed drug as well as a rapid solubilization rate [31–33]. The dissolution behavior of TA from bSD pellets in comparison with TA powders and TA/PVP physical mixtures (PMs) is shown in Fig. 1.

TA powders exhibited a poor dissolution rate with only 5% drug dissolved after 120 min (Fig. 1A). These data could be related to its crystalline patterns shown by DSC (Fig. 5A). TA/PVP PMs presented a slight improvement in the dissolution properties in comparison with the pure drug (Fig. 1B and C). These results suggested that PVP itself might act as a weak solubilizer in PMs, contributing to the limited increase in drug dissolution, as well as in preventing drug aggregation [34]. Nevertheless, as shown from Fig. 1, it was evident that bSD pellets exhibited faster dissolution rates than TA powders and corresponding PMs. At the TA/PVP ratio of 1/4, approximately 60% of TA was dissolved within 60 min, which was about a 10-fold increase compared with TA powders (Fig. 1A and F). Similar results have been reported by Bley et al. [33] and Visser et al. studies [20]. The increase in dissolution of TA from bSD pellets might be attributed to many factors such as a reduction in the drug crystallinity and molecular dispersion of drug in PVP. The dissolution rate of TA from bSD pellets varied depending on the ratio of TA/PVP, along with the increasing ratios, the dissolution rate of TA correspondingly increased (Fig. 1D–F). The fact was consistent with Thybo et al. [35] and Paradkar et al. studies [36], which indicated that the increase in dissolution rate can be

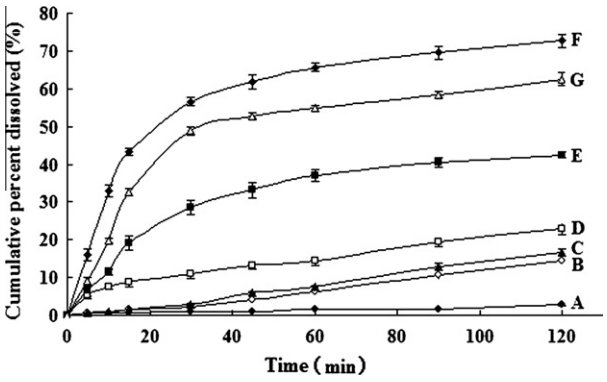

Fig. 1. The dissolution profiles of (A) TA, (B) TA/PVP (1/2, PMs), (C) TA/PVP (1/6, PMs), and bSD pellets at different TA/PVP ratios of (D) TA/PVP (1/1), (E) TA/PVP (1/2), (F) TA/PVP (1/4) and (G) TA/PVP (1/6). Each point represents the mean ± SD ($n = 3$).

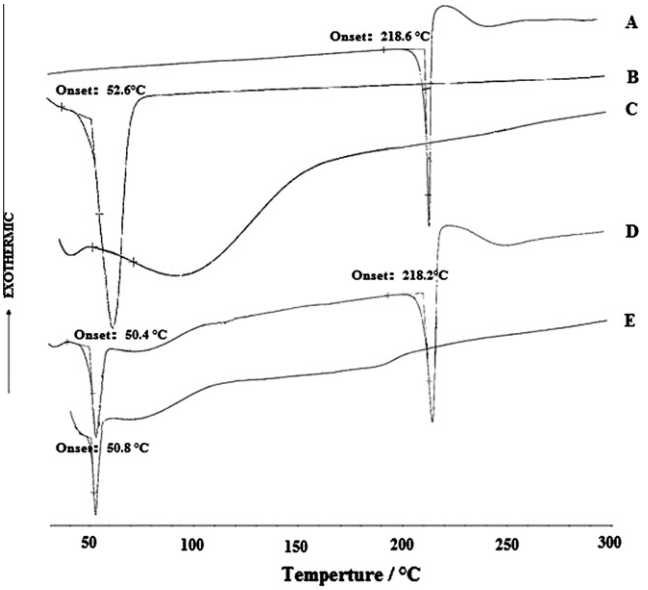

Fig. 5. DSC curves of TA (A), poloxamer 188 (B), PVP (C), TA/PVP/ poloxamer 1/4/1 PMs (D) and tSDs at TA/PVP/poloxamer 188 ratio of 1/4/1 (E).

achieved by increasing the amount of PVP in SDs. With increasing to 1/6 (Fig. 1G), the dissolution rate of TA reversely declined compared to bSD pellets with TA/PVP ratio of 1/4. The result was similar to that found by Akbuga et al. [37], Ford et al. [38] and Pandit et al. [39] studies. It was suggested that the decrease in dissolution rate of SDs containing higher PVP proportions might be caused by the leaching out of the carrier during dissolution, which could form a concentrated layer of PVP solution around the drug particles and thus slow down the migration of the dissolved drug particles to the bulk of the dissolution medium.

3.2.2. Effects of poloxamer 188 on dissolution of TA

In order to promote further dissolution behavior of TA, a novel ternary solid dispersion systems consisting of TA, PVP and poloxamer 188 were investigated.

As shown in Fig. 2, when comparing tSD pellets and bSD pellets, a faster dissolution rate of TA was observed after the incorporation of poloxamer 188. Pellets containing TA/PVP/poloxamer 188 (1/4/1) provided a 1.67-fold increase in dissolution relative to the binary TA/PVP (1/4) systems without poloxamer 188 incorporated after 60 min. The results can be inferred that, during dissolution

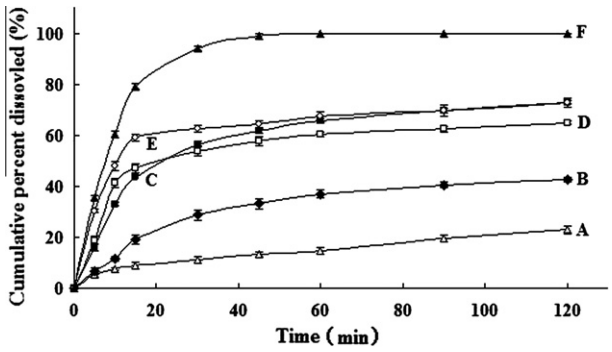

Fig. 2. The dissolution profiles of bSD pellets at different TA/PVP ratios of (A) TA/PVP (1/1), (B) TA/PVP (1/2), (C) TA/PVP (1/4) and tSD pellets at different TA/PVP/poloxamer 188 ratios of (D) TA/PVP/poloxamer 188 (1/1/1), (E) TA/PVP/poloxamer 188 (1/2/1) and (F) TA/PVP/poloxamer 188 (1/4/1). Each point represents the mean ± SD (n = 3).

of bSD pellets, an inevitable high drug concentration formed in the near vicinity of the dissolving pellets especially when PVP dissolved at a much faster rate, resulting in uncontrollable recrystallization and formation of large crystals which obviously dissolve slowly, and similar results were reported by van Drooge et al. [18]. Hence, as a countermeasure, in the ternary solid dispersion systems, the incorporation of poloxamer 188 may generate a high surfactant concentration, which just could enhance the solubility of dissolved drug, prevent agglomeration of drug into large globules or particles in the aqueous environment and thus effectively inhibit crystallization during dissolution [22,40–45]. On the other hand, poloxamer 188 present on surface could decrease the surface tension dissolution between media and drug particle and hence bringing about additional wettability and solubilizing effect [41]. Moreover, drug could be dispersed into mostly submicron size particles [46] under the action of poloxamer 188, which could further facilitate the dissolution.

3.2.3. Effects of incorporated poloxamer 188 content on dissolution of TA

Effect of the content of poloxamer 188 in ternary systems on TA dissolution was investigated by comparing three different ratios of TA/PVP/poloxamer 188 in Fig. 3.

Judging from the dissolution results in Fig. 3, the dissolution rate of TA in ternary systems was directly proportional to the content of poloxamer 188. However, the dissolution rate of TA reversely decreased at higher level (after 1/4/2 ratio) (Fig. 3B), which was also observed by Ghebremeskel et al. [47] and Chambliss et al. studies [48]. Poloxamer 188 always existed in an amphiphilic structure, which has the properties to self-assemble into micelles in aqueous solution when the concentration was above the critical micellar concentration (CMC). TA might be entraped by micelles formed by poloxamer 188, which may become an obstacle to the further dissolution.

Based on the above dissolution test, the optimum formulation was ascertained to be TA, PVP and poloxamer 188 at the ratio of 1/4/1, and the inclusion of surfactant and drug/carrier proportion were the prerequisites to the enhancement of dissolution.

3.3. Characterization of TA tSD pellets

3.3.1. Visualization by SEM

Observed under SEM, the TA tSD pellets prepared by the optimum formulation were spherical (Fig. 4A). Concave pores were uniformly distributed on the surface under larger magnifications (Fig. 4B and C), which probably be produced during the process of spray solution volatilization from the surface of solid dispersion pellets. The cross-section view indicated multilayers of compact coating of solid dispersion around the sugar pellets core (Fig. 4D). Pellets surface can be increased with these concave holes

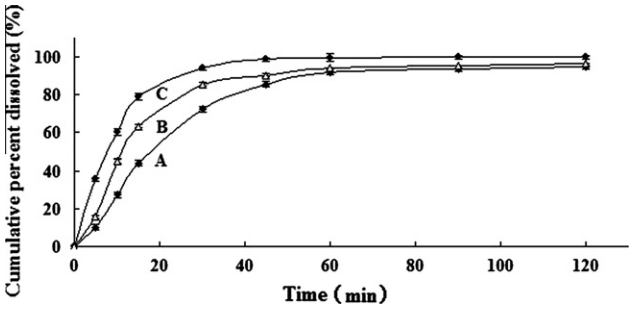

Fig. 3. The dissolution profiles of tSD pellets at different TA/PVP/poloxamer 188 ratios of (A) TA/PVP/poloxamer 188 (1/4/0.5), (B) TA/PVP/poloxamer 188 (1/4/2) and (C) TA/PVP/poloxamer 188 (1/4/1). Each point represents the mean ± SD (n = 3).

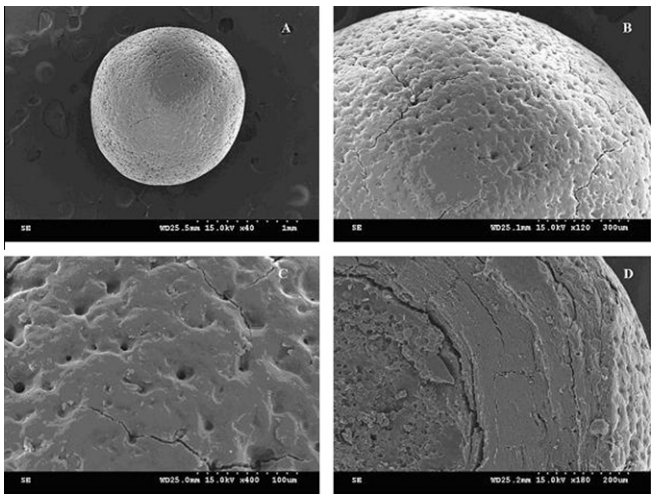

Fig. 4. Scanning electron micrographs of the surface (A: 40×; B: 120×; C: 400×) and cross-section (D: 180×) of the TA solid dispersion pellets prepared with TA/PVP/poloxamer 188 ratio of 1/4/1.

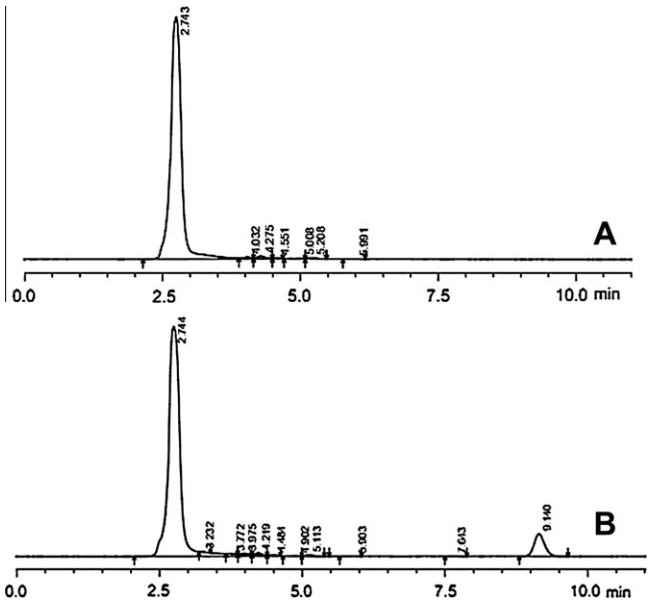

Fig. 6. Typical HPLC chromatograms of Tanshinone II A in rabbit plasma: (A) blank plasma and (B) plasma sample after oral administration of TA tSD pellets at a dose 30 mg kg^{-1} of TA. Retention time of TA was approximately 9.140 min.

which would facilitate the inward water penetration and dissolution of the incorporated drug.

3.3.2. DSC

The DSC thermograms of TA tSD pellets, physical mixtures, combined carriers and TA are presented in Fig. 5. The DSC curve of TA (Fig. 5A) exhibited a sharp endothermic peak at 218.6 °C corresponding to its melting point, indicating its crystalline nature, followed by an exothermic peak at 228.6 °C, which may be ascribed to the decomposition of TA. The endothermic peak of poloxamer 188 appeared at 52.6 °C (Fig. 5B). During scanning of PVP, a broad endotherm ranging from 70 to 130 °C (Fig. 5C) was observed indicating the loss of water due to the extremely hygroscopic nature of PVP polymers. Concerning the physical mixture of TA/PVP/poloxamer 188 (1/4/1), the drug endothermic peak was found at 218.2 °C. The PVP dehydration peaks and the poloxamer 188 endothermic peak were also present, as if the thermogram was the sum of those of the components analyzed separately. This thermogram indicated that the absence of interaction between TA and the carriers in PMs and a solid dispersion could not be obtained by simple blending of the drug and carriers (Fig. 5D). As can be seen in Fig. 5A and E, the complete disappearance of the endothermic peak corresponding to TA was observed in TA/PVP/ poloxamer 188 (1/4/1) solid dispersions (Fig. 5E), suggesting that the drug may be present as microcrystalline, nanocrystalline or amorphous state [49]. Interestingly, the decomposition exothermic peak also disappeared, which may suggest stabilization effect of TA by PVP/poloxamer 188 solid dispersion (Fig. 5E). It is concluded that the formation of solid dispersions by solvent evaporation in the fluidized-bed system is feasible.

3.4. In vivo performance

TA in plasma could be completely separated under analytical conditions, and no significant matrix effect was observed for the analytes in the plasma samples (Fig. 6). The standard curve was found to be linear, $y = 175264x + 324.5$ ($n = 3$, $r = 0.9976$, where x is the concentration of TA, and y is the corresponding peak area in the 0.005–0.5 μg mL^{-1} range). The limit of quantification was 10 ng mL^{-1}. Intraday and interday variabilities were below 10%. The results attained from the relative recoveries of high, middle and low concentrations were 107.31 ± 22.56%, 104.63 ± 10.74% and 98.29 ± 5.35%, respectively. All of the absolute recoveries were above 80%, with all RSD less than 10%, which were within the acceptable limits to meet the guidelines for bioanalytical methods.

The pharmacokinetic data of four formulations (e.g., TA, its PMs, tSD and bSD pellets) were processed following an open one-compartment model. The plasma concentrations of TA versus time obtained after oral administration were shown in Fig. 7. The main pharmacokinetic parameters were listed in Table 2.

Compared with TA, TA tSD pellets, TA bSD pellets and PMs always presented remarkably larger C_{max} and AUC_{0-t} ($p < 0.01$) in the order of TA tSD pellets > TA bSD pellets > PMs and earlier T_{max} ($p < 0.01$) in the order of TA tSD pellets < TA bSD pellets < PMs. Regardless of coexisting with carrier by physical mixing or highly dispersing, TA in PMs, bSD and tSD systems entirely exhibited faster absorption rate and higher bioavailability under the action of molecular dispersion of drug in PVP and/or the solubilization and wetting effect of poloxamer 188. Although TA tSD pellets and its PMs contained the same ingredients, in the ternary solid dispersions systems, drug incorporated in the hydrophilic carrier may be molecularly dispersed or may present as nanocrystals or

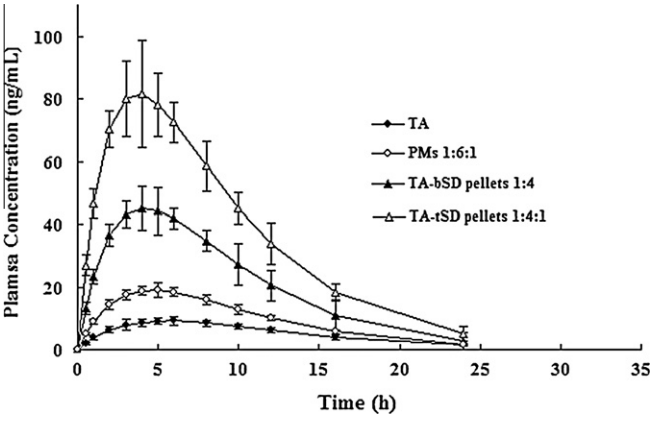

Fig. 7. Mean plasma concentration–time curve of TA in rabbits after oral administration of TA tSD pellets, TA bSD pellets, PMs and TA equivalent to 30 mg kg^{-1} of TA ($n = 6$), respectively. Values are mean ± SD ($n = 6$/group/time point).

Table 2
The main pharmacokinetic parameters of TA after oral administration of TA tSD pellets, TA bSD pellets, PMs or TA in rabbits ($n = 6$) in a dose of 30 mg/kg.

Parameters	T_{max} (h)	C_{max} (ng mL^{-1})	AUC_{0-t} (ng h mL^{-1})	$AUC_{0-\infty}$ (ng h mL^{-1})	Relative bioavailability (%)
TA	5.52 ± 0.738[a]	9.16 ± 0.172[a]	140.43 ± 38.928[a]	150.52 ± 45.404[a]	–
PMs	4.65 ± 0.226[d]	19.03 ± 2.386[d]	232.03 ± 56.234[d]	240.78 ± 39.456[d]	165
bSDpellets	4.15 ± 0.456[c]	45.08 ± 6.954[b]	509.84 ± 106.248[b]	525.16 ± 123.813[b]	363
tSD pellets	3.80 ± 0.398	82.13 ± 17.046	899.02 ± 142.386	929.30 ± 150.527	640

Data are expressed as mean ± SD ($n = 6$).
[a] $P < 0.01$ are statistical significances with TA versus TA tSD pellets, TA bSD pellets or PMs.
[b] $P < 0.01$ are statistical significances with TA tSD pellets versus TA bSD pellets.
[c] $P < 0.05$ are statistical significances with TA tSD pellets versus TA bSD pellets.
[d] $P < 0.01$ are statistical significances with TA tSD pellets versus PMs.

amorphous nanoparticles [49], which could be further strengthened with the participation of surfactant [22,50]. Therefore, the combined carriers could enormously expand the contact area with the gastrointestinal tract, resulting in an accelerated absorption rate and improved bioavailability [49,51]. In addition, the inclusion of surfactant in ternary solid dispersions can further increase the wettability and porosity properties of drugs [47,52], which can be another strong contribution to the enhancement of drug relative bioavailability. Compared with TA bSD pellets, TA tSD pellets presented obviously larger AUC_{0-t}, C_{max} ($p < 0.01$) and earlier T_{max} ($p < 0.05$), which can be attributed to the rapid absorption rate and improved bioavailability resulted from the incorporation of poloxamer 188 by avoiding drug recrystallization, enhancing additional wettability and solubilizing effect and potentiating their dissolution.

4. Conclusion

In this study, it was found that the addition of poloxamer 188 to pellets containing PVP-based solid dispersions was an alternative strategy to improve the dissolution behavior of TA. As compared to the use of PVP alone, the combination of PVP and poloxamer 188 in the prepared tSD pellets significantly improved the absorption rate and oral bioavailability of TA. Moreover, the fluid-bed coating technique can be used to deposit solid dispersions on sugar pellets and may find application in the manufacturing and scaling-up of solid dispersion formulations in the future.

Acknowledgements

This study is financially supported by the major project of National Science and Technology of China for new drugs development (No. 2009ZX09310-004) and Jiangsu Province Ordinary College and University innovative research programs (No. CX10B-374Z). Thanks to JCR, ISP and BASF corporations for providing the excipients and blank sugar pellets.

References

[1] L. Zhou, Z. Zuo, M.S.S. Chow, Danshen: an overview of its chemistry pharmacology, pharmacokinetics, and clinical use, J. Clin. Pharmacol. 45 (2005) 1345.
[2] D.D. Sun, H.C. Wang, X.B. Wang, Y. Luo, Z.X. Jin, Z.C. Li, G.R. Li, M.Q. Dong, Tanshinone II A: A new activator of human cardiac KCNQ1/KCNE1 (I_{Ks}) potassium channels, Eur. J. Pharmacol. 590 (2008) 317–321.
[3] J. Gao, G. Yang, R. Pi, R. Li, P. Wang, H. Zhang, K. Le, S. Chen, P. Liu, Tanshinone II A protects neonatal rat cardiomyocytes from adriamycin-induced apoptosis, Transl. Res. 151 (2008) 79–87.
[4] J. Li, G. Wang, P. Li, H. Hao, Simultaneous determination of tanshinone II A and cryptotanshinone in rat plasma by liquid chromatography–electrospray ionisation–mass spectrometry, J. Chromatogr. B 826 (2005) 26–30.
[5] C.D. Li, J.P. Liu, Z. Z Zeng, Y.X. Zheng, Study on the solubility and permeability of tanshinone II A and on the excipients increasing the solubility and permeability, Lishizhen Med. Materia Med. Res. 19 (2008) 1724–1726.
[6] L. Wang, X. Jiang, W. Xu, C. Li, Complexation of tanshinone II A with 2-hydroxypropyl-β-cyclodextrin: effect on aqueous solubility, dissolution rate, and intestinal absorption behavior in rats, Int. J. Pharm. 341 (2007) 58–67.
[7] H. Hao, G. Wang, N. Cui, J. Li, L. Xie, Z. Ding, Pharmacokinetics, absorption and tissue distribution of tanshinone II A solid dispersion, Planta Med. 72 (2006) 1311–1317.
[8] H. Hao, G. Wang, N. Cui, J. Li, L. Xie, Z. Ding, Identification of a novel intestinal first pass metabolic pathway: NQO1 mediated quinone reduction and subsequent glucuronidation, Curr. Drug Metba. 8 (2007) 137–149.
[9] J. Yuan, S. Mao, Q. Shen, S. Hou, Y. He, Influence of solid dispersion technique combination on dissolution of tanshinone II A, J. Chinese Mater. Med. 34 (2009) 685–689.
[10] R. Gandhi, C. Lal Kaul, R. Panchagnula, Extrusion and spheronization in the development of oral controlled-release dosage forms, Pharm. Sci. Technol. Today 2 (1999) 160–170.
[11] N. Zerrouk, C. Chemtob, P. Arnaud, S. Toscani, J. Dugue, In vitro and in vivo evaluation of carbamazepine-PEG 6000 solid dispersions, Int. J. Pharm. 225 (2001) 49–62.
[12] G.F. Palmieri, F. Cantalamessa, P. Di Martino, C. Nasuti, S. Martelli, Lonidamine solid dispersions: in vitro and in vivo evaluation, Drug Dev. Ind. Pharm. 28 (2002) 1241–1250.
[13] S. Lee, K. Nam, M.S. Kim, S.W. Jun, J.S. Park, J.S. Woo, S.J. Hwang, Preparation and characterization of solid dispersions of itraconazole by using aerosol solvent extraction system for improvement in drug solubility and bioavailability, Arch. Pharm. Res. 28 (2005) 866–874.
[14] M. Arias, J. Gines, J. Moyano, A. Rabasco, The application of solid dispersion technique with D-mannitol to the improvement in oral absorption of triamterene, J. Drug Target 2 (1994) 45–51.
[15] A. Serajuddin, Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs, J. Pharm. Sci. 88 (1999) 1058–1066.
[16] C.W. Lin, T.M. Cham, Effect of particle size on the available surface area of nifedipine from nifedipine–polyethylene glycol 6000 solid dispersions, Int. J. Pharm. 127 (1996) 261–272.
[17] O.I. Corrigan, Mechanisms of dissolution of fast release solid dispersions, Drug Dev. Ind. Pharm. 11 (1985) 697–724.
[18] D. Van Drooge, W. Hinrichs, H. Frijlink, Anomalous dissolution behaviour of tablets prepared from sugar glass-based solid dispersions, J. Control Rel. 97 (2004) 441–452.
[19] W.I. Higuchi, N.A. Mir, S.J. Desai, Dissolution rates of polyphase mixtures, J. Pharm. Sci. 54 (1965) 1405–1410.
[20] M.R. Visser, L. Baert, G. Klooster, L. Schueller, M. Geldof, I. Vanwelkenhuysen, H. de Kock, S. De Meyer, H.W. Frijlink, J. Rosier, Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240, Eur. J. Pharm. Biopharm. 74 (2010) 233–238.
[21] X. Wang, A. Michoel, G. Van den Mooter, Solid state characteristics of ternary solid dispersions composed of PVP VA64, Myrj 52 and itraconazole, Int. J. Pharm. 303 (2005) 54–61.
[22] H.N. Joshi, R.W. Tejwani, M. Davidovich, V.P. Sahasrabudhe, M. Jemal, M.S. Bathala, S.A. Varia, A. Serajuddin, Bioavailability enhancement of a poorly water-soluble drug by solid dispersion in polyethylene glycol–polysorbate 80 mixture, Int. J. Pharm. 269 (2004) 251–258.
[23] R. Jachowicz, E. Nürnberg, B. Pieszczek, B. Kluczykowska, A. Maciejewska, Solid dispersion of ketoprofen in pellets, Int. J. Pharm. 206 (2000) 13–21.
[24] T. Vasconcelos, B. Sarmento, P. Costa, Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs, Drug Discov. Today 12 (2007) 1068–1075.
[25] H.O. Ho, H.L. Su, T. Tsai, M.T. Sheu, The preparation and characterization of solid dispersions on pellets using a fluidized-bed system, Int. J. Pharm. 139 (1996) 223–229.
[26] I. Ghebre-Sellassie, R.H. Gordon, M.B. Fawzi, R.U. Nesbitt, Evaluation of a high-speed pelletization process and equipment, Drug Dev. Ind. Pharm. 11 (1985) 1523–1541.
[27] W. Zhang, J. Liu, X. Liu, Z. Chen, Stealth tanshinone II A-loaded solid lipid nanoparticles: effects of poloxamer 188 coating on in vitro phagocytosis and in vivo pharmacokinetics in rats, Acta Pharm. Sinica 44 (2009) 1421–1428.
[28] Y. Zhang, M. Huo, J. Zhou, S. Xie, PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel, Comput. Meth. Prog. Biol. 99 (2010) 306–314.

[29] Z. Yong, A data analysis program in pharmacokinetics base on microsoft excel-development and validation of PKSlover 1.0, J. Math. Med. 20 (2007) 58–61.
[30] P. Srinarong, J. Faber, M. Visser, W. Hinrichs, H. Frijlink, Strongly enhanced dissolution rate of fenofibrate solid dispersion tablets by incorporation of superdisintegrants, Eur. J. Pharm. Biopharm. 73 (2009) 154–161.
[31] M. Yoshioka, B.C. Hancock, G. Zografi, Inhibition of indomethacin crystallization in poly (vinylpyrrolidone) coprecipitates, J. Pharm. Sci. 84 (1995) 983–986.
[32] S.L. Shamblin, G. Zografi, Enthalpy relaxation in binary amorphous mixtures containing sucrose, Pharm. Res. 15 (1998) 1828–1834.
[33] H. Bley, B. Fussnegger, R. Bodmeier, Characterization and stability of solid dispersions based on PEG/polymer blends, Int. J. Pharm. 390 (2010) 165–173.
[34] Y. Kawabata, K. Yamamoto, K. Debari, S. Onoue, S. Yamada, Novel crystalline solid dispersion of tranilast with high photostability and improved oral bioavailability, Eur. J. Pharm. Sci. 39 (2010) 256–262.
[35] P. Thybo, B.L. Pedersen, L. Hovgaard, R. Holm, A. Müllertz, Characterization and Physical Stability of Spray Dried Solid Dispersions of Probucol and PVP-K30*, Pharm. Dev. Technol. 13 (2008) 375–386.
[36] A. Paradkar, A.A. Ambike, B.K. Jadhav, K. Mahadik, Characterization of curcumin-PVP solid dispersion obtained by spray drying, Int. J. Pharm. 271 (2004) 281–286.
[37] J. Akbuga, A. Gursoy, E. Kendi, The preparation and stability of fast release furosemide-PVP solid dispersion, Drug Dev. Ind. Pharm. 14 (1988) 1439–1464.
[38] J.L. Ford, The effect of particle size on some in vitro and in vivo properties of indomethacin–polyethylene glycol 6000 solid dispersion, Drug Dev. Ind. Pharm. 12 (1986) 1777–1793.
[39] J.K. Pandit, B.K. Khakurel, In vitro and in vivo evaluation of some fast release dosage forms of hydrochlorothiazide, Drug Dev. Ind. Pharm. 10 (1984) 1709–1724.
[40] S. Okonogi, S. Puttipipatkhachorn, Dissolution improvement of high drug-loaded solid dispersion, AAPS Pharm. Sci. Technol. 7 (2006) 148–153.
[41] P. Mura, J. Moyano, M. González-Rodríguez, A. Rabasco-Alvaréz, M. Cirri, F. Maestrelli, Characterization and dissolution properties of ketoprofen in binary and ternary solid dispersions with polyethylene glycol and surfactants, Drug Dev. Ind. Pharm. 31 (2005) 425–434.
[42] P. Mura, M. Faucci, A. Manderioli, G. Bramanti, P. Parrini, Thermal behavior and dissolution properties of naproxen from binary and ternary solid dispersions, Drug Dev. Ind. Pharm. 25 (1999) 257–264.
[43] K.R. Morris, G.T. Knipp, A. Serajuddin, Structural properties of polyethylene glycol–polysorbate 80 mixture, a solid dispersion vehicle, J. Pharm. Sci. 81 (1992) 1185–1188.
[44] M. Aldén, J. Tegenfeldt, E. Sj kvist, Structure of solid dispersions in the system polyethylene glycol–griseofulvin with additions of sodium dodecyl sulphate, Int. J. Pharm. 83 (1982) 47–52.
[45] M. Aldén, J. Tegenfeldt, E.S. Saers, Structures formed by interactions in solid dispersions of the system polyethylene glycol–griseofulvin with charged and non charged surfactants added, Int. J. Pharm. 94 (1993) 31–38.
[46] A. Serajuddin, P.C. Sheen, D. Mufson, D.F. Bernstein, M.A. Augustine, Physicochemical basis of increased bioavailability of a poorly water-soluble drug following oral administration as organic solutions, J. Pharm. Sci. 77 (1988) 325–329.
[47] A.N. Ghebremeskel, C. Vemavarapu, M. Lodaya, Use of surfactants as plasticizers in preparing solid dispersions of poorly soluble API: selection of polymer–surfactant combinations using solubility parameters and testing the processability, Int. J. Pharm. 328 (2007) 119–129.
[48] W.G. Chambliss, R.W. Cleary, R. Fischer, A.B. Jones, P. Skierkowski, W. Nicholes, A.H. Kibbe, Effect of docusate sodium on drug release from a controlled-release dosage form, J. Pharm. Sci. 70 (1981) 1248–1251.
[49] C. Leuner, J. Dressman, Improving drug solubility for oral delivery using solid dispersions, Eur. J. Pharm. Biopharm. 50 (2000) 47–60.
[50] A. Serajuddin, P.C. Sheen, D. Mufson, D.F. Bernstein, M.A. Augustine, Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water-soluble drug from solid dispersions, J. Pharm. Sci. 77 (1988) 414–417.
[51] D. Bikiaris, G.Z. Papageorgiou, A. Stergiou, E. Pavlidou, E. Karavas, F. Kanaze, M. Georgarakis, Physicochemical studies on solid dispersions of poorly water-soluble drugs: evaluation of capabilities and limitations of thermal analysis techniques, Thermochim. Acta 439 (2005) 58–67.
[52] G. Van den Mooter, I. Weuts, T. De Ridder, N. Blaton, Evaluation of Inutec SP1 as a new carrier in the formulation of solid dispersions for poorly soluble drugs, Int. J. Pharm. 316 (2006) 1–6.