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Original Research Paper

Blends of hydrophobic and swelling agents in the swelling layer in the preparation of delayed-release pellets of a hydrophilic drug with low MW: Physicochemical characterizations and in-vivo evaluations

Chang You [a,b], *Xuedan Liang* [a], *Jiabei Sun* [a,c], *Lin Sun* [a], *Yitao Wang* [a], *Tianyuan Fan* [d], *Ying Zheng* [a,*]

[a] *State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao SAR, China*

[b] *Beijing Institute for Drug Control, Beijing, China*

[c] *National Institute for Food and Drug Control, Beijing, China*

[d] *Department of Pharmaceutics, School of Pharmaceutical Sciences, Peking University, Beijing, China*

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ABSTRACT

In this study, a hydrophobic material, ethylcellulose, which was used as its aqueous suspension Surelease®, was combined with a swelling agent as the swelling layer to prepare delayed-release pellets for Danshensu, which is a hydrophilic drug with low MW. A rupturable, delayed-release pellet consists of a drug core, a swelling layer containing a swelling agent (cross-linked sodium carboxymethyl cellulose) with a hydrophobic agent (Surelease®), and a controlled layer composed by an insoluble, water-permeable polymeric coating (aqueous ethylcellulose dispersions) was developed in a fluidised bed. Results showed that blending Surelease® into the swelling layer could effectively extend the release of Danshensu from the pellets, which may be attributed to the slowed swelling rate by reduction of water penetration and improvement of mechanical integrity of the swelling layer. Drug in the delayed pellets showed sustained release in beagle dogs after oral administration with comparable in-vivo exposure to the uncoated drug pellets. In conclusion, blends of hydrophobic and swelling agents in the swelling layer in double-membrane pellets could achieve a delayed drug-release profile in vitro, as well as delayed and sustained absorption in vivo for highly soluble, low-MW drug. The present

Abbreviations: DDS, drug delivery system; HPLC, high-performance liquid chromatography; DAD, diode array detector; HPMC, hydroxypropyl methylcellulose; L-HPC, low-substituted hydroxypropyl cellulose; CMC-Na, sodium carboxymethyl cellulose; CC-Na, cross-linked sodium carboxymethyl cellulose; SEM, scanning electron microscope; MRT, mean residence time; AUC, area under concentration-time curve.

* *Corresponding author*. Institute of Chinese Medical Sciences, University of Macau, 3/F, Rm 204A, Block 3, Av. Padre Tomás Pereira, S.J. Taipa, Macao SAR, China. Tel: +86 853 83974687; fax: +86 853 28841358.
E-mail address: yzheng@umac.mo (Y. Zheng).

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study highlighted the potential use of a delayed-release system for other hydrophilic, low-MW drugs to meet the formulation requirements for chronopharmacological diseases.

1. Introduction

Chronopharmacology is the study of how the effects of drugs vary with biological timing and endogenous periodicities, which is relevant to many diseases, such as ischaemic heart disease and hypertension, which are frequently occurred before dawn [1]. The circadian rhythms showed that drug pharmacokinetics and the effects of therapies changed with the time of drug administration. Therefore, maintaining an adequate blood concentration during the episodes of a disease not only improves efficacy but also reduces the side effects of the drug [2,3].

Delayed-release drug delivery system (DDS) is a chronopharmacology- and hominal physiology-based DDS, mainly applied for the treatment of diseases exhibiting circadian rhythms, for instance, ischaemic heart disease, asthma and intestinal diseases [4]. Patients may take drug before going to sleep at around 10 pm, and active components in the medicine will be released after several hours delay (e.g., 2–4 h) and take effect at night during the episodes of the disease. As a multi-particulate DDS, delayed-release pellets are receiving increasing interest. This administration mode offers many advantages when compared with normal single-unit delivery systems, including good reproducibility, generally short gastric residence time and low risk of dose dumping [5]. There are several approaches to prepare delayed-release pellets, such as pH dependent enteric coating and double-membrane coating. Most of the double-membrane based pellets systems contain a drug reservoir surrounded by a swelling and a controlled layer, which will erode, dissolve, or rupture [6]. However, the model drugs used in double-membrane based pellets are mostly western drugs, and few studies have been performed to produce the delayed release of low-MW (e.g., ~200) and water-soluble drugs in Chinese Medicine.

Danshensu (MW 198) is a hydrophilic and quality control marker of Fufang Danshen Dripping Pill, which has been officially listed in the Chinese pharmacopoeia [7] and has been approved by FDA for phase III clinical trial. The main pharmacological activities of Danshensu include dilating coronary arteries [8], inhibiting platelet aggregation [9], improving microcirculation [10] and protecting the myocardium from reperfusion injury of the ischaemic heart [11]. After oral administration of Danshensu to rats, Danshensu was quickly absorbed and rapidly eliminated from the systemic circulation, with a T_{max} of 38 min and a $t_{1/2}$ of 45 min [12]. Moreover, its oral bioavailability in rat is only about 11% regardless being delivered as pure compound or herbal extraction [13,14]. As two widely selling products of Danshen in China, Fufang Danshen Tablet and Fufang Danshen Dripping Pill are immediately released dosage forms (q.i.d) and could not meet the needs of chronotherapeutic treatment for ischaemic heart disease.

As a low-toxic, non-allergic and non-irritant polymer, ethylcellulose is a widely used polymer for film coating in modified-release pellets [15]. In recent years, the use of organic solutions has been replaced by the aqueous dispersion Surelease® with the aim of avoiding the toxicity of organic solvents to the environment, reducing viscosity and loading higher polymer contents [16–18]. Surelease® is a well-developed plasticised aqueous dispersion designed specifically for modified-release and taste-masking applications. It is an aqueous dispersion mainly containing plasticisers and ethylcellulose. Due to its hydrophobic property, ethylcellulose was always used as a barrier for water penetration in film and matrix formation [19].

To take the advantage of the pellets as well as to meet the requirement of the chronopharmacology for cardiovascular disease, this study aimed to take Danshensu as a model drug and develop its delayed-release pellets using double-membrane system. A rupturable, pulsatile-release pellet consists of (i) a drug core (Danshensu was used as model drug); (ii) a swelling layer comprising a swelling agent (CC–Na) and a hydrophobic agent (Surelease®, aqueous ethylcellulose dispersions); and (iii) a controlled layer consisting of an insoluble, water-permeable polymeric coating (Surelease®). The performance of the pellets was characterised in terms of morphology, drug release profile and in vivo pharmacokinetics in beagle dogs.

2. Materials and methods

2.1. Materials

Sodium Danshensu was obtained from Xi'an Hongson Biotechnology Co. Ltd., China. Sodium Danshensu and caffeic acid standard substance were obtained from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China. Suglet® CP-507 (500–700 μm, NP Pharm, France) was used as the core material for the construction of the spherical, blank and drug-loaded pellets. Surelease® E-7-19050 (for experimental use only) was obtained from Colorcon, Shanghai, China. Other excipients of the delayed-release pellets were hydroxypropyl methylcellulose (HPMC, Colorcon, Shanghai, China), calcium carboxymethyl cellulose (CMC-Ca, NICHIRIN, Japan), cross-linked sodium carboxymethyl cellulose (CC–Na, Ac-Di-Sol SD-711, FMC, USA), L-hydroxypropyl cellulose (L-HPC, Shin Etsu, Japan), calcium carboxymethyl cellulose and croscarmellose sodium (CMC-Ca). All chemical reagents used were at analytical or HPLC grade.

Table 1 – Summary of fluid bed's parameters during coating for each layer.

Operating condition	Set values			
	Layering of drug	Sealed coating with HPMC	Surelease with CC–Na as swelling layer	Controlled layer of Surelease
Before coating				
Preheating to (°C)	41	41	42	41
Coating	0.4	0.3	0.3	0.4
Airflow (Bar)				
Inlet temperature (°C)	45	45	50	45
Spray rate (g/min)	0.8	1.0	1.5	1.0
Spray pressure (Bar)	0.85	0.95	1.25	0.9
Product temperature (°C)	38–40	37–39	35–37	38–39
After coating				
Curing in oven	–	–	60 °C for 2 h	60 °C for 2 h
Mesh size (mesh)	30	30	20	20

2.2. Preparation of Danshensu delayed-release pellets

2.2.1. Drug core

Sodium Danshensu (0.4 g) was dissolved in 10 g HPMC solution 2% (w/w). Sugar cores (50 g) (Suglet®) were placed into the fluidised bed (Mini-Glatt 4, Glatt GmbH, Germany) and pre-heated to 41 °C. The coating conditions are summarised in Table 1. During processing, the spraying rate and inlet air temperature were fine tuned to maintain the product temperature at 38 ~ 40 °C. After coating, the sticking pellets were removed using mesh in each stage (Table 1). Drug loading rate was calculated by comparing the drug amount extracted from the pellets with the initial drug added.

2.2.2. Seal-coating of Danshensu-loaded cores with HPMC

The Danshensu-loaded cores were seal-coated with HPMC 3% (w/w) before film-coating to prevent the potential interaction between the drug and the polymers in the swelling layer. HPMC (1.5 g) was dissolved completely in 48.5 g of water, and the solution was sprayed onto 50 g of sodium Danshensu-loaded pellets in a fluidised bed. Then, 30-mesh was used to remove the sticking pellets.

2.2.3. Optimisation of the swelling layer

Firstly, four swelling materials (i.e., HPMC, CMC-Ca, L-HPC and CC–Na) were screened. The four polymers were individually mixed with 3% HPMC solution at a ratio of 1:20 (w/w) for the coating, where HPMC was the binding agent when combined with the other polymers. Then in-vitro release profiles were evaluated in terms of three levels of weight gain of CC–Na in the swelling layer of the pellets. Furthermore, the influence of blending Surelease with CC–Na was studied. Surelease (25%, w/w) was added into 500 g water and mixed for approximately 15 min, and then CC–Na was added into the Surelease solution and mixed well. Keeping the weight gain of CC–Na constant at 20%, suspensions containing CC–Na and Surelease at ratios of 1:1, 1:3 and 1:5 (w/w) were prepared by adjusting the amount of Surelease (Table 2). The suspension was used to coat 50 g of the drug-loaded and sealed pellets. After coating, the pellets were heat-cured in the oven at 60 °C for 2 h to form complete film. Coating efficiency was calculated by comparing the solid amount of the coating materials and the total weight of the coated pellets.

2.2.4. Controlled layer coated with surelease

Surelease was also used as the material in the controlled layer. Surelease (25%, w/w) was diluted to 15% (w/w) by adding distilled water while stirring for approximately 15 min. Then, 50 g of pellets were coated with diluted Surelease as the controlled layer, where its thickness was equivalent to three different theoretical weight gains of 20%, 25% and 30%. Heat-curing was performed by using the same method as described in swelling layer preparation, and the actual content of sodium Danshensu in the pellets was determined by HPLC. Coating efficiency was calculated by comparing the solid amount of the coating materials and the total weight of the coated pellets.

2.3. In-vitro release test

The in-vitro release profile of Danshensu from the pellets was determined according to the paddle method described in Chinese Pharmacopoeia 2010 [7]. The volume of the medium was 375 ml of 0.1 N HCl for the first 2 h, and then 125 ml of 0.2 N sodium phosphate solution was added to adjust the pH from 1.2 to 6.8 for the remaining 10 h. The temperature was 37 ± 0.5 °C with mechanical stirring at 100 rpm. Pellets containing 10 mg of Dashensu were filled in a gelatin capsule for the release study. Samples (3.0 ml) were withdrawn from each vessel at predetermined intervals and replaced with the same volume of water to maintain the total volume. The samples were analyzed by HPLC to determine the drug content. Drug release data from 10% to 80% in the release profile were selected for fitting using Zero-order, first-order and Higuchi equations.

Table 2 – Samples of pellets with different swelling agents.

Swelling agent	Swelling layer weight gain	Controlled layer weight gain
HPMC	20%	20%
CMC-Na	20%	20%
L-HPC	20%	20%
CC–Na	10%, 20%, 30%	20%
CC–Na – Surelease	40% (1:1), 80% (1:3), 120% (1:5)	20%

2.4. Optical microscopic observation

To observe the process of drug release in the release media, a few pellets with the optimised formula were taken out at 0, 2, 3, 5 and 7 h, respectively from the release test. The pellets were dried carefully with a tissue and observed by an optical microscope.

2.5. Scanning electron microscopy (SEM)

Two types of delayed-release pellets containing different swelling layers (CC–Na alone or with Surelease at a weight ratio of 1:5) were studied. The pellets were mechanically cleaved transversely and sputtered with gold. The surface of the pellets and swelling layers were examined by scanning electron microscopy (SEM, S-3700, HITACHI) at 10 kV.

2.6. Water vapour permeability of cast films

Three different levels of polymer dispersions containing CC–Na and Surelease at ratios of 1:1, 1:3 and 1:5 (w/w) were cast onto a Teflon plate. The films were dried in an oven at 40 °C and heat-cured at 60 °C for 2 h. The water vapour permeabilities of the three different films were determined according to the ASTM guideline E 96-00 using the desiccant method [20]. The thickness of the film was measured by a micrometre 8 times at different places and the results were averaged. The film specimen covered the open mouth of a weighing bottle (~3 cm inner diameter and 3 cm depth) containing 15 g desiccant, which was accurately weighed and placed into a chamber maintained at 75% RH using a saturated sodium chloride solution stored at 25 °C. The bottles were weighed after 48 h to determine the weight gain, and the water–vapour permeability (P) was calculated using the following two equations:

$$\mathrm{WVT} = \frac{G}{tA} \tag{1}$$

$$\mathrm{P} = \frac{\mathrm{WVT}}{S} \times (R_1 - R_2) \times d \tag{2}$$

where G is the weight change, t is the time during which G gained, A is the test area (weighing bottle mouth area), S is the saturation vapour pressure at the test temperature, R_1 is the relative humidity in the test chamber, R_2 is the relative humidity inside the bottle (0% for the desiccant method) and d is the average thickness of the film [21].

2.7. In-vivo study

2.7.1. Oral administration to beagle dogs

The animal experiment was approved by the internal ethics committee in University of Macau (Macau SAR, China). Animal experiments are conducted in full compliance with national regulatory principles. The in-vivo performance of the delayed release pellets with optimized formulation (see Section 2.2.4) or drug-loaded uncoated pellets (see 2.2.1) were evaluated in beagle dogs. Three male beagle dogs (weighing 10–14 kg, provided by Guang Dong Medical and Experimental Animal Centre) were used. The dogs were fasted for 12 h with free access to water before the drug administration. Danshensu delayed-release pellets were filled in gelatin capsules and orally administered to beagle dogs with water at 9 mg/kg of Danshensu in a single dose. A blood sample of 2 ml was withdrawn from the forelimb vein of each of the dogs at predetermined times and centrifuged at 14,000 rpm for 5 min to separate the plasma. A sample of 0.5 ml of plasma was collected, and 0.1 ml 10% HCl containing 5% ascorbic acid was added to improve the stability of Danshensu in the plasma. The plasma samples were kept frozen at −20 °C until they were analyzed by HPLC. After a 7-day wash out period following the last administration, the drug-loaded uncoated pellets were administered according to the same method as described above.

2.7.2. HPLC Determination of Danshensu

For the in-vivo pharmacokinetic study, the plasma concentration of Danshensu in the beagle dog was determined by HPLC according to the method described in our previous study [22]. A reverse-phase C_{18} column (250 × 4.6 mm, 5 μm, Agilent Technologies, Inc., Palo Alto, CA, USA) was used with UV detection at 280 nm. The mobile phase was a 0.5% phosphoric acid/methanol 82:12 (v/v) mixture and the flow rate was 1.0 ml/min. Good linearity in the range from 0.10 μg/ml to 8.00 μg/ml was obtained with the limit of quantity (LOQ) of 0.04 μg/ml. The accuracy and precision of this method were acceptable for quantitative analysis.

2.7.3. Pharmacokinetic parameters

The maximum drug concentration (C_{max}) and the time to maximal plasma drug concentration (T_{max}) were obtained as directly measured values. The areas under the plasma concentration–time curve ($AUC_{0\text{-}24\ h}$) and mean retention time (MRT) were calculated by WinNolin software (WinNonlin version 5.0.1) by assuming a non-compartmental model for drug absorption and distribution. The lag time of drug appearance in plasma (T_{lag}) was defined as the time corresponding to the first appearance of Danshensu in the plasma.

2.8. Statistical analysis

The pharmacokinetic parameters were presented as the mean ± SD. and Student's t-test was employed for statistical comparison. A P-value of less than 0.05 was considered as significant.

3. Results and Discussion

3.1. Formulation optimisation

Agglomeration rate and coating efficiency were chosen as the key indexes to evaluate the pellets coating performance. The agglomeration rates under all the coating conditions in this study were below 2%. Drug loading rate was ~90% when using 2% HPMC as the binding agent, and further increased HPMC concentration could not improve drug loading rate. Therefore, the following experiment used 2% of HPMC as the binding agent for drug coating. When CC–Na, L-HPC, CMC-Ca were mixed with 3% HPMC as the swelling layer, the coating

efficiency were only around 70%–80%, which may be due to that the coating materials were suspension and lack of the plasticizer. After mixing the CC–Na with Surelease and HPMC, the coating efficiency of the swelling layer were higher than 90%. Moreover, the coating efficiency of the Surelease as the controlled release layer were all above 90%, all of which indicting the good coating efficiency for different layers on fluidized bed.

Preliminary experiments showed that ~70% of the total drug was sustained released in 12 h from the pellets coated by a single Surelease layer with 10% weight gain (data not shown). With an increase in the weight gain of Surelease, the rate of drug release decreased, and only approximately 35% of the drug could be released in 12 h at 30% weight gain of Surelease. These results indicate that with an increase in Surelease weight gain, the rate of drug release from the pellets decreased, which may be due to that Surelease film controls the rate of water diffusion into the pellets. If the pellets have thicker Surelease film, more time will be required for water diffusion into the pellets. Therefore, to ensure that water could diffuse into the pellets slowly, at least 20% of Surelease weight gain was required in the controlled layer.

Using 20% Surelease as the controlled layer, four swelling materials, including HPMC, CMC-Ca, L-HPC and CC–Na, were screened. The first three swelling agents are super-disintegrates that can expand hundreds times after absorbing water, and HPMC is a polymer hydrogel. Results showed the swelling ability of these four materials followed the order CC–Na > L-HPC > CMC-Na > HPMC. The swelling ability of the first two materials was strong enough to ensure the complete release of Danshensu after 12 h. However, as CMC-Na and HPMC only have limited swelling ability, the release of Danshensu using these agents is more likely to be extended release rather than immediate release. The drug-release profile of the pellets using HPMC as the swelling layer displayed sustained release because the swelling layer of HPMC gelatinises instead of swelling when it absorbs water. Therefore, CC–Na was chosen as the swelling agent in the swelling layer to prepare the Danshensu delayed-release pellets in the following sections.

With 20% Surelease as the controlled layer, the effect of weight gain of the swelling layer CC–Na on the release of Danshensu is studied. Only 10% weight gain of CC–Na has enough swelling ability to crack the controlled layer. However, even when the weight gain of CC–Na was increased to 30%, no significant delay could be observed, indicating that the lag time of the delayed-release pellets could not be achieved simply by enhancing the weight gain of CC–Na.

The results above showed that delayed-release pellets with 2–6 h lag time could not be realised by only adjusting the weight gain of the controlled layer and the swelling layer with the formula used above. Therefore, Surelease, a hydrophobic material, was blended with the swelling agent in the swelling layer to decrease the swelling rate of CC–Na. Three types of delayed-release pellets were prepared to produce different ratios of Surelease in the swelling layer and keeping weight gain of the CC–Na constant at 20%. The ratios of CC–Na and Surelease were 1:1, 1:3 and 1:5 (w/w), with a weight gain of the controlled layer of 20% in all samples. The release profiles of the delayed-release pellets are shown in Fig. 1. When the ratio was 1:1, the lag time of Danshensu was 1.5 h. When the ratio

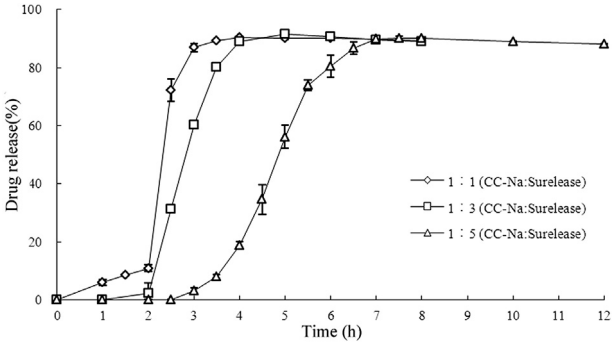

Fig. 1 – Effect of different CC–Na and Surelease ratios in the swelling layer on the release of Danshensu maintaining 20% CC–Na weight gain in the swelling layer and 20% of Surelease weight gain as the controlled layer ($n = 3$).

was increased to 1:3 and 1:5, the lag time extended to 2 h and 3.5 h, respectively. After the lag time, the Danshensu was released from the pellets quickly, and the drug was completely released within 3 h. This result indicated that the lag time of the delayed-release pellets could be increased effectively by adding hydrophobic Surelease in the swelling layer. By increasing the ratio of Surelease in the swelling layer, pellets with a longer lag time could be prepared. Then, Danshensu could be released from the pellets rapidly and completely. Finally, the ratio between CC–Na and Surelease was set at 1:5.

After optimising the swelling layer, the effect of the weight gain of Surelease in the controlled layer on drug release was investigated. As shown in Fig. 2, when the weight gain of the controlled layer was 20%, the lag time of the delayed release was 3.5 h. With the weight gain increased to 25%, the lag time was extended to 5 h. When the weight gain was further increased to 30%, the lag time was extended to 6 h, but the drug release rate was significantly decreased, and it took 12 h for the drug to be completely released from the pellets. To balance the lag time and drug release rate, Surelease at 20% was used as the controlled layer.

Overall, the optimized formulation of the swelling layer was set as blending CC–Na and Surelease at a weight ratio of 1:5 with total weight gain of 120%, and the Surelease was used as the controlled layer with 20% of weight gain with Danshensu loading at 4 mg/g for in-vitro characterization and 12 mg/g for in-vivo pharmacokinetic study.

3.2. *The fitting of drug release equation*

The in-vitro drug-release profile of the delayed-release pellets optimised previously was fitted by three different equations, as shown in Table 3. The drug release of the delayed-release pellets followed a zero-order equation, indicating that the release of drug maintained at a constant rate.

3.3. *Optical microscopic observation*

Optical microscopic pictures of the pellets are shown in Fig. 2. At 0 h and 2 h, the surface of the delayed-release pellets was

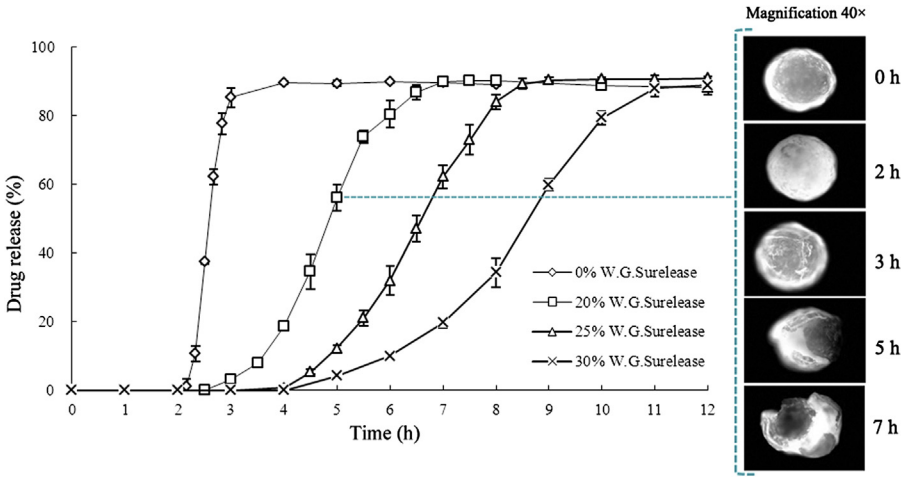

Fig. 2 – Effect of weight gain of Surelease as the controlled layer on drug release when the weight ratio of CC–Na to Surelease is 1:5 and the total weight gain in the swelling layer is 120% ($n = 3$).

still intact. Some rifts appeared at 3 h with a small amount of Danshensu starting to release. At 5 h, the pellets busted and the core containing Danshensu could make contact with the medium more completely. The pellets were broken completely at 7 h, when the core was totally exposed to the medium and the Danshensu was completely released.

3.4. *Scanning electron microscopy*

SEM results demonstrated that the thickness of the swelling layer and the controlled layer is around 100 μm and 30 μm, respectively in the optimized formulation (data not shown). Fig. 3 showed SEM images of the cross-sections of two types of pellets with different swelling layers. In the pellets of swelling layer containing CC–Na and Surelease (Fig. 3A and B) a homogenous and uniform polymer film was observed. The compact film could become a barrier between the water and the drug, preventing the Danshensu from dissolving in the water before the delayed-release time. Using only CC–Na did not lead to the formation of compact film (Fig. 3C and D). However, the film was loose and porous. Therefore, there was a potential risk that the water-soluble drug of a low molecular weight could dissolve into the water, which would penetrate into the pellets through the pores. This result may explain why blending hydrophobic Surelease into the swelling layer can extend the lag time of water-soluble drugs of low molecular weight.

Table 3 – The fitted models for drug release from delayed release pellets.

Model	Equation	Equation fitted	R^2
Zero order	$Mt/M_\infty = k\,t$	$Mt/M_\infty = 0.3799\,t$	0.9867
First order	$\ln(M_\infty - Mt) = -k\,t + M$	$\ln(M_\infty - Mt) = -0.76\,t + 11.946$	0.9426
Higuchi	$M\,t/M_\infty = k\,t^{1/2}$	$\ln(M_\infty - Mt) = 1.5904\,t^{1/2}$	0.9803

3.5. *Water vapour Permeability of cast films*

The water vapour permeability of cast films at ratios of 1:1, 1:3 and 1:5 (w/w. CC–Na: Surelease) is $(3.64 \pm 0.48) \times 10^{-5}$, $(2.97 \pm 0.33) \times 10^{-5}$, $(1.83 \pm 0.04) \times 10^{-5}$ g/Pa·h·m, respectively. The water vapour permeability of the film composed of CC–Na and Surelease at a ratio of 1:5 is almost half of those at a ratio of 1:1, indicating that an increase in the amount of Surelease results in a decrease in the water vapour permeability of the film and less water could pass through the film. Due to the hydrophobicity of Surelease, blending Surelease into the material can prevent water from effectively passing through the film.

Due to the main ingredient in Surelease, ethyl cellulose, blending hydrophobic Surelease can effectively improve the physical structure of the swelling film. It is easier to form a film with ethyl cellulose than with CC–Na. Therefore, CC–Na can form a better compact film with the help of ethyl cellulose. The efficiency of the coating and utilisation of coating materials could also be improved by plasticiser. Water vapour permeability showed that a higher ratio of Surelease in the swelling layer led to a longer lag time because more Surelease in the swelling layer required a longer time for water to permeate into the swelling layer. A longer lag time must be achieved to reach enough force for the swelling agent to rupture the controlled layer and release the drug completely.

3.6. *In-vivo pharmacokinetic evaluation*

The performance of the drug delayed-release system was evaluated in-vivo. In this investigation, the Danshensu delayed-release pellets were evaluated by measuring the plasma concentration profiles of Danshensu after oral administration of coated and uncoated pellets. The mean concentration-time curves for the Danshensu delayed-release pellets and the uncoated pellets in three beagle dogs are illustrated in Fig. 4, and all the pharmacokinetic parameters were calculated using WinNolin and are listed in Table 4.

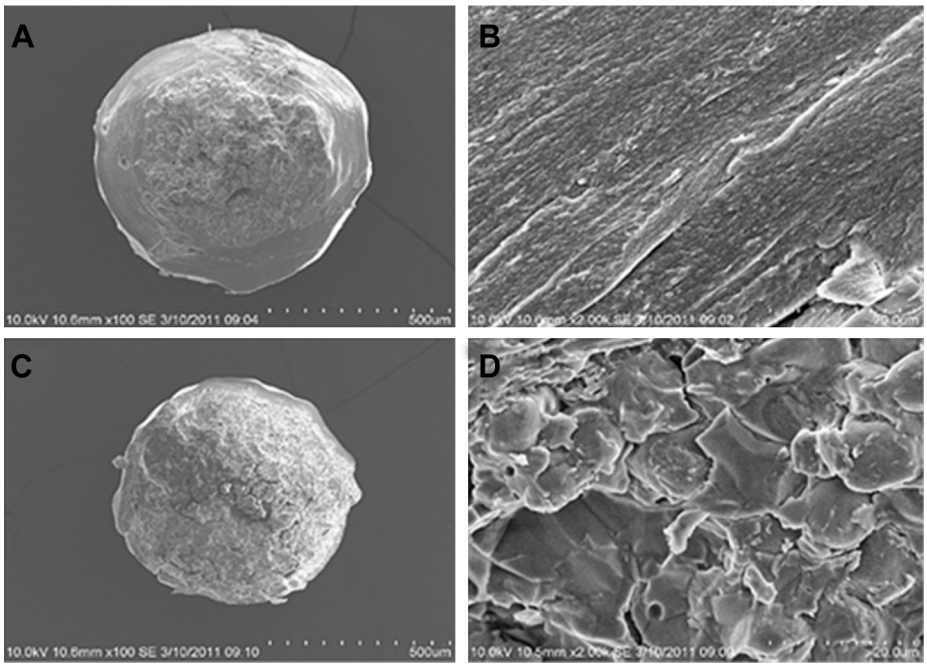

Fig. 3 – SEM graphs of Danshensu delayed-release pellets: (A) cross-section of the pellets using CC–Na and Surelease at the ratio of 1:5 (w/w) with 120% weight gain as the swelling layer; (B) magnification of the swelling layer composed of CC–Na and Surelease; (C) cross-section of the pellets only using CC–Na as the swelling layer; (D) magnification of the swelling layer of CC–Na.

When the uncoated pellets were administered to the beagle dogs, Danshensu appeared in plasma immediately without any lag time, and then the blood concentration of Danshensu sharply increased with a T_{max} of 1.08 ± 0.38 h. While the delayed-release pellets showed a 1.5 ± 0.41 h lag time, and the T_{max} was significantly extended to 3.33 ± 0.58 h, $T_{1/2}$ and MRT also significantly increased from 1.90 ± 1.40 h to 2.51 ± 0.33 h and from 1.50 ± 0.43 h to 6.36 ± 1.04 h, respectively. These results indicated that the double-layer delayed-release pellets developed could retard drug release in-vivo and maintain the drug in the body for a longer time. In addition, C_{max} significantly decreased from 3.42 ± 0.72 μg/ml to 0.74 ± 0.28 μg/ml and AUC remained unchanged. These results suggest that after the lag time of drug release, Danshensu was released in a sustained manner rather than immediately from the pellets.

Although the pulsatile release in vitro is obvious, the plasma drug concentration-time plot showed significantly sustained absorption and elimination profiles. This apparent disparity may be due to several reasons. One possible reason may be that only limited gastric fluid are available in beagle dog's stomach and intestine, while there is plenty of medium used in the in-vitro release experiments. Therefore, swelling layer of pellets in the GI tract may not absorb enough water and break up the controlled layer as quickly as those under in-

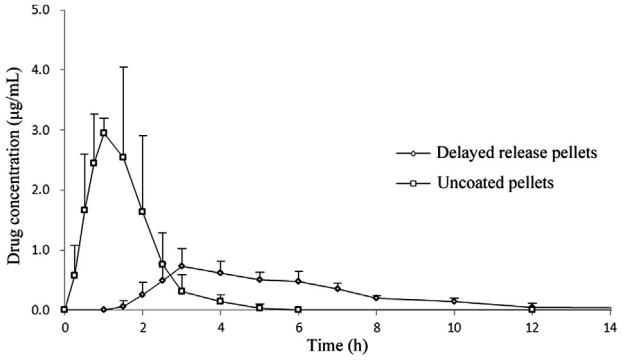

Fig. 4 – Plasma concentration-time profiles of Danshensu after oral administration of drug-loaded pellets or drug loaded delayed-release pellets of Danshensu at 9 mg/kg in beagle dogs ($n = 3$). Pellets were coated using CC–Na and Surelease at the ratio of 1:5 (w/w) with 120% weight gain as the swelling layer, and Surelease layer at 20% weight gain as the controlled layer at drug loading of 12 mg/g.

Table 4 – Pharmacokinetic parameters of Danshensu pellets after oral administration in beagle dog ($n = 3$).

Parameters	Uncoated pellets [22]	Delayed release pellets
T_{lag} (h)	0.00 ± 0.00	1.50 ± 0.41*
T_{max} (h)	1.08 ± 0.38	3.33 ± 0.58*
$T_{1/2}$	0.65 ± 0.26	2.51 ± 0.33*
MRT (h)	1.50 ± 0.43	6.36 ± 1.04*
C_{max} (μg/ml)	3.42 ± 0.72	0.74 ± 0.28*
AUC (μg*h/ml)	5.22 ± 1.48	3.91 ± 1.16

All the data are presented in the form of Mean ± SD.
* Statistically significant difference compared to uncoated pellets ($P < 0.05$).

vitro conditions, which will lead to the sustained absorption and elimination. Another possibility may be that the absorption of Danshensu occurs mainly in the small intestine [23]. While this delayed-release system has a lag time, it might cause some pellets to reach the colon before the entire drugs are completely released. And it is expected that the absorption of Danshensu in the colon is poor, leading to a reduced absorption and elimination.

Although the delayed-release pellets showed a much longer T_{lag} and higher MRT than the uncoated pellets, their T_{lag} was shorter than the value obtained from the in-vitro drug-release studies. The variation between the in-vitro and in-vivo results may be caused by two reasons. Firstly, the developed pellets are both time- and pH-dependent, as the swelling ability of CC–Na depends on the pH value (data not shown). The higher pH value, the stronger swelling ability of CC–Na is [24]. Secondly, there are many physiological differences between humans and dogs [25], such as the gastric emptying time (0.19 h) and intestinal transit time (3.40 h), which are both much shorter in dogs than those in humans. The in-vitro release conditions (e.g., time and pH) used in the present study simulated human GI conditions, which are not consistent with the in-vivo GI conditions of a beagle dog. Therefore, the delayed-release pellets would contact the high pH value environment earlier in beagle dogs than those in the release test, where the CC–Na could swell faster, which may contribute to the shorter lag time in the in-vivo pharmacokinetic study. Furthermore, the gastric mechanical destructive abilities of the beagle dog (3.2 N) are stronger than those of humans (1.5 N) [26], so some pellets may be more easily damaged in beagle dogs, which may be another reason for the shorter lag time in the in-vivo study. All of these factors led to the poor correlation between the in-vitro and in-vivo performances of the coated pellets. To better simulate human conditions, GI physiology regulated dogs, whose gastric pH, gastric emptying time and small intestine transit time are almost identical to those of the human may be considered for future work. Murata et al. [25] regulated beagle dogs' GI physiology by a combined treatment of intramuscular injection of pentagastrin (10 mg/kg, bid, with a 45 min interval) with intravenous injection of atropine sulphate (0.02 mg/kg). They have demonstrated good correlation between in-vitro and in-vivo studies.

4. Conclusions

To achieve a delayed drug-release profile for highly soluble low-MW drugs, blends of hydrophobic and swelling agents in the swelling layer are required. The lag time was controlled by the coating level of the swelling and controlled layer, as well as the ratio between the hydrophobic agent and the swelling agent in the swelling layer. Blending Surelease in the swelling layer retards water penetration and enhances the mechanical structure of the film by slowing the swelling rate of CC–Na. The drug-release mechanism controlled by the rupturing of the outer layer was confirmed by microscopic observations of pellet behaviour during release, where the drug release followed a zero-order equation. In-vivo results confirmed the delayed and sustained absorption of Danshensu in beagle dogs for the coated pellets. The present study highlighted the potential use of the delayed-release system for other hydrophilic low-MW drugs to meet the formulation requirements for chronopharmacological diseases.

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