

## Design and Evaluation of pH-Independent Pulsatile Release Pellets Containing Isosorbide-5-mononitrate

Yao LIU, Songqing LIU,\* and Qing DAI

Department of Pharmacy, Southwest Hospital, Third Military Medical University; 29 Gao Tan Yan Street, Chongqing 400038, China. Received September 7, 2008; accepted October 27, 2008; published online October 29, 2008

**A three-layered, pH-independent pulsatile release pellets system containing isosorbide-5-mononitrate (ISMN) was studied. The process of the heart disease such as angina has a close relationship to the chronobiology, which gives rise to the need of a pulsatile drug deliver system for the anti-anginal drug. In this study, pellets containing ISMN were firstly prepared as the core, and then layered with a swelling layer followed by an water-insoluble control layer. The core pellets were formulated with microcrystalline cellulose (MCC) and lactose, and were prepared by extrusion-spheronization. The preparation was optimized by Box–Behnken experimental design, when taking the MCC/lactose ratio as well as the operating conditions of extrusion-spheronization as variables. The experimental results demonstrated the relationships between formulation, operation and properties of the product, and meanwhile provided optimized values for the parameters. The core pellets were coated by a fluidized bed coater, and pellets with various coating types and coating levels were studied by *in vitro* dissolution tests. The effects of both swelling layer and control layer on the lag time and the drug release time were studied, in order to predetermine the lag time and release time. The pellets were also evaluated *in vivo* by studying the pharmacokinetics after oral administration in beagle dogs. The pellets achieved a lag time of 4.1 h *in vivo*, which had a good consistency with the *in vitro* results, and the relative bioavailability was nearly 100% comparing to the normal tablets.**

**Key words** pulsatile release pellet; isosorbide-5-mononitrate; Box–Behnken experimental design

In recent years, people have got better understanding on the circadian rhythms of many diseases, such as angina, hypertension, bronchial asthma and rheumatic disease.<sup>1)</sup> The process of these diseases has a close relationship to the day–night rhythm.<sup>2,3)</sup> Take angina for example, the fatal heart attack is prone to stroke in the midnight due to its circadian rhythm. This will give rise to the failure of survival and brings new challenge to the drug delivery systems.

The typical oral sustained release systems keep the *in vivo* drug concentration in the therapeutic level for a prolonged period of time, and this is necessary but not sufficient for treatment of circadian rhythm diseases. Therefore, a novel delivery system designed for this kind of diseases, called pulsatile system, has drawn much interest in recent years.<sup>4,5)</sup> Pulsatile drug delivery systems can be characterized by a predetermined lag time after which the drug release is triggered automatically or by the change of physiological circumstances.<sup>6)</sup> The drug release pattern corresponds to the requirement of circadian rhythm treatment, so as to achieve the ideal therapeutic effect and minimize the adverse effects.

In this study, a three-layer pulsatile release pellets system for delivery of isosorbide-5-mononitrate (ISMN) was designed and evaluated. ISMN is the major active metabolite of isosorbide dinitrate (ISDN), an anti-anginal drug. ISMN has the similar pharmacological action as ISDN and is clinical used as a vasodilator. The major mechanism of its anti-anginal action is primarily based on an increase in venous capacitance leading to a decreased return of blood to the heart. ISMN has a complete oral absorption without first-pass effect, and its bioavailability is nearly 100% with little individual variation. But drug tolerance has appeared in more than 60% of patients due to uncontrolled dose and interval of administration, therefore the best therapeutic efficacy can hardly be achieved for this population. Generally, angina pectoris tends to stroke in midnight at which time taking medi-

cine is inconvenient; and on the other hand, normal sustained release dosage forms will raise the rate of drug tolerance. The objective of this study is to develop an ISMN pulsatile release pellets (ISMN-PRP) system to fit the midnight episode of angina pectoris. The three-layered pulsatile release pellets system was composed of a drug containing core, an inner swelling layer and an outer controlling coating, in order to achieve a pulsatile drug release after oral administration at a predetermined lag time.

### Experimental

**Materials** Microcrystalline cellulose, MCC (Avicel® PH101, Asahi Kasei Corporation, Japan); lactose anhydrous (Wyndale®, Lactose Company of New Zealand, New Zealand); hydroxypropylmethylcellulose, HPMC (Methocel® E5LV, Colorcon, Dartford, England); low-substituted hydroxypropylcellulose, L-HPC (LH21, ShinEtsu, Japan); aqueous dispersion of ethylcellulose (Surelease® E-7-19010, Colorcon, U.S.A.); Croscarmellose Sodium, CCNa (Primellose®, DMV, Holland); isosorbide-5-mononitrate, ISMN (Lunan-Beite Pharm., Shandong, China). All other reagents were of analytical grade.

**Preparation of Drug Containing Cores** The cores of the pellets were prepared by an extrusion-spheronization machine (E-100/S-450, Yingge Drying Machine Co., Ltd., Chongqing, China). MCC, lactose anhydrous and ISMN were mixed sufficiently and was added a certain amount of 6% water solution of HPMC to make a wet mass which was subsequently extruded through a 0.8 mm screen by the extruder. The extruded material was spheronized by the spheronizator to form pellets cores with appropriate dimeters. After spheronization, the obtained pellets cores were dried in a fluidized bed (CPM300, Guangxia Drying Equipment Co., Ltd., Chongqing, China) for 1 h. The inlet air temperature was 60 °C and the intake flow rate was 80 m<sup>3</sup>·h<sup>-1</sup>. Then the pellets were sieved and the moiety ranging in 20–24 mesh were collected to conduct the coating process.

**Optimization of Formulation** The formulation and operating condition of extrusion-spheronization were optimized by Box–Behnken experimental design<sup>7,8)</sup> to obtain pellets with best micromeritic properties. The factors and responses for investigation are shown in Table 1. When the drug content was constant, the ratio of MCC and lactose ( $X_1$ ) was the major factor affecting the pellets' micromeritic properties, such as circularity ( $Y_1$ ) and friability ( $Y_2$ ). The spheronization speed ( $X_2$ ) and spheronization time ( $X_3$ ) had major influences on the final product. The degree of pellet circularity was deter-

\* To whom correspondence should be addressed. e-mail: songqingliu@hotmail.com

Table 1. Factors and Responses Investigated in the Optimization Experimental Design

Factors	Levels		
	-1	0	1
$X_1$ MCC/lactose (w/w)	3	5	7
$X_2$ spheronization speed (rpm)	300	450	600
$X_3$ spheronization time (min)	2	4	6
Response	Constraints		
$Y_1$ circularity ( $\varphi_c$ )	Maximized		
$Y_2$ friability (%)	Minimized		

mined by computer imaging technique. Briefly, about 500 pellets were stuck on a plain paper, and its image was scanned into computer. The mean projected area ( $A$ ) and mean projected circumference ( $L$ ) of the tested pellets were obtained by analyzing the image. Then the degree of circularity ( $\varphi_c$ ) was calculated as:

$$\varphi_c = \frac{\pi D_H}{L}, \quad D_H = \sqrt{\frac{4A}{\pi}}$$

$D_H$  was the mean projected area diameter (Heywood diameter).

The degree of friability was evaluated by the weight loss of pellets before and after a rotating disk test. Briefly, about 100 g of pellets was centrifugated at 900 r·min<sup>-1</sup> for 5 min in a rotating disk (150 mm radius, horizontally rotating) of a centrifuging granulator (MedUnion Research Institute, Liaoning, China). After the test, the pellets were sieved and the weight loss from the crush was obtained to estimate the friability as follow:

$$\text{friability (\%)} = \frac{\text{weight loss}}{\text{initial weight}} \times 100\%$$

**Coating of the Pellets** The pellet cores were coated with layer materials using a fluidized bed,<sup>8)</sup> the inlet air temperature was adjusted to 38 °C and the air-blower frequency 30 Hz. The pellets were coated with an 8% (w/w) L-HPC solution in 96% (v/v) ethanol or a 15% (w/w) CCNa solution in water, respectively, as the swelling layer. The spray rate was 1.4 ml·min<sup>-1</sup>. The primarily coated pellets were dried at 38 °C for 15 min, and then coated with ethylcellulose aqueous dispersion (Surelease<sup>®</sup> E-7-19010) as control layer. The operating conditions were same as the swelling layer coating as described above. Before coating, Surelease<sup>®</sup> was diluted with water to 15% (w/w) solid content. The coating time was controlled to achieve a specific weight gain. After coating, the pellets were kept drying in the fluidizing bed at 38 °C for 2 h.

**Drug Release from the Uncoated Cores** The *in vitro* drug release was studied by a dissolution apparatus (type RC-3B, electric factory of Tianjin University), according to the basket method for drug dissolution test described in Chinese Pharmacopoeia 2005, appendix XC. Uncoated drug containing cores containing 5 mg of ISMN were processed in the baskets. The drug release was investigated in 0.1 M HCl, pH 6.8 PBS or distilled water, each 900 ml, respectively. The paddle rotating rate was 100 rpm and temperature was 37±0.5 °C. All the experiments were carried out in triplicates. Five milliliters of samples were withdrawn at predetermined time points, filtered by 0.45 μm membranes and determined by HPLC. HPLC conditions were as follows: a Kromasil<sup>®</sup> C<sub>18</sub> column (250 mm×4.6 mm, 5 μm) was used; The mobile phase was a mixture of methanol and water (30/70, v/v); the flow rate was 1.0 ml·min<sup>-1</sup> and the column temperature was 30 °C. The retention time of ISMN was 6.288 min.

**Drug Release from Pulsatile Pellets** Pellets containing 5 mg of ISMN were processed in the baskets apparatus described above. The dissolution media was 675 ml of 0.1 M HCl for the first 2 h, and afterward an additional of 225 ml of 0.2 M Na<sub>2</sub>PO<sub>4</sub> solution was added to convert the pH to 6.8 in the following hours. The paddle rotating rate was 100 rpm and temperature was 37±0.5 °C. The sampling and analysis methods were as described above. In this study, the lag time ( $t_{10}$ ) was defined as the intersected point on the time axis when 10% of the drug was released. The release time ( $t_{10-75}$ ) was defined as the difference between  $t_{75}$  and  $t_{10}$ .

Table 2. Observed Responses of Box–Behnken Experimental Design

No.	$X_1$ MCC/ lactose (w/w)	$X_2$ Spheronization speed (rpm)	$X_3$ Spheronization time (min)	$Y_1$ Circularity ( $\varphi_c$ )	$Y_2$ Friability (%)
1	7	450	6	0.882	0.736
2	5	600	6	0.875	0.492
3	7	600	4	0.834	1.016
4	5	450	4	0.887	0.467
5	5	450	4	0.871	0.440
6	7	450	2	0.820	0.956
7	5	600	2	0.794	0.544
8	3	600	4	0.839	0.514
9	7	300	4	0.818	1.085
10	5	450	4	0.877	0.481
11	3	450	6	0.898	0.363
12	3	300	4	0.824	0.560
13	5	300	2	0.756	0.773
14	3	450	2	0.841	0.511
15	5	300	6	0.840	0.596

**Pharmacokinetics in Beagle Dogs** The animal study was approved and performed in accordance with the guidelines of the Institutional Animal Ethics Committee. Pharmacokinetics data of ISMN pulsatile release pellets were evaluated, when taking commercially available ISMN tablets (Xinkang<sup>®</sup>, Lunan Beite Pharm. Co., Shangdong, China; 20 mg/tablet) as reference, in beagle dogs. Six beagle dogs (0.5–1.0 years old, 10.8±1.0 kg) were randomly divided into two groups and fasted for 12 h before dosing. The dogs were given ISMN tablets or ISMN pulsatile release pellets (filled in capsules) in a single dose of 40 mg of ISMN per dog. The study was performed in a double-cycle, cross over design. Two milliliters of blood was sampled through an indwelling catheter in the hind limb medial subcutaneous vein. The time points of sampling were predose (0 h), 0.33, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12 and 24 h for pulsatile pellets group, or predose (0 h), 0, 2, 3, 3.5, 4, 4.33, 4.67, 5, 5.33, 5.67, 6, 6.5, 7, 8, 10, 12 and 24 h for reference group, respectively.

The blood samples anticoagulated by heparin were centrifuged (3000 rpm, 3 min) to separate the plasma. Plasma samples were stored at -20 °C. The ISMN concentration in the plasma samples was analyzed by GC-ECD method.<sup>9)</sup> Prior to analysis, 500 μl of plasma sample was added 100 μl of saturated K<sub>2</sub>CO<sub>3</sub> solution and 50 μl of ISDN (isosorbide dinitrate) solution (2 μg·ml<sup>-1</sup>, as internal standard), followed by mixing with 4 ml of diethyl ether/*n*-hexane (4/1, v/v) mixture. After extraction by vortex, the organic layer was collected and evaporated to dryness under a nitrogen flow, and re-dissolved in 50 μl of ethyl acetate for GC analysis. The GC conditions were as follows: The column was a phenyl methyl silicone capillary column BP-5 (30 m×0.53 mm, 0.5 μm). Nitrogen was used as the carrier gas at a constant flow-rate of 30 ml·min<sup>-1</sup>. The oven temperature was maintained at 140 °C for 1.0 min after injection, then increased to 190 °C at 50 °C/min, and finally holding at 190 °C for 8.0 min. The injector and detector temperatures were maintained both at 250 °C.

## Results and Discussion

**Preparation of Pellet Cores and Its Optimization** In the preparation process, both the formulation and preparing conditions would affect the micromeritic properties of the final product, and they might have relationships with each other. Therefore, a 3-factor Box–Behnken experimental design (3 center points, 15 runs) was carried out for optimizing both the formulation and preparation factors, which were integrated in one experimental group. The circularity and friability of the pellets were selected as target response of the optimization. The experimental design and its observed responses are shown in Table 2 and Figs. 1 and 2.

It can be observed from the plot figure that the circularity ( $Y_1$ ) was mainly affected by spheronization speed ( $X_2$ ) and spheronization time ( $X_3$ ), while MCC/lactose ratio ( $X_1$ ) and

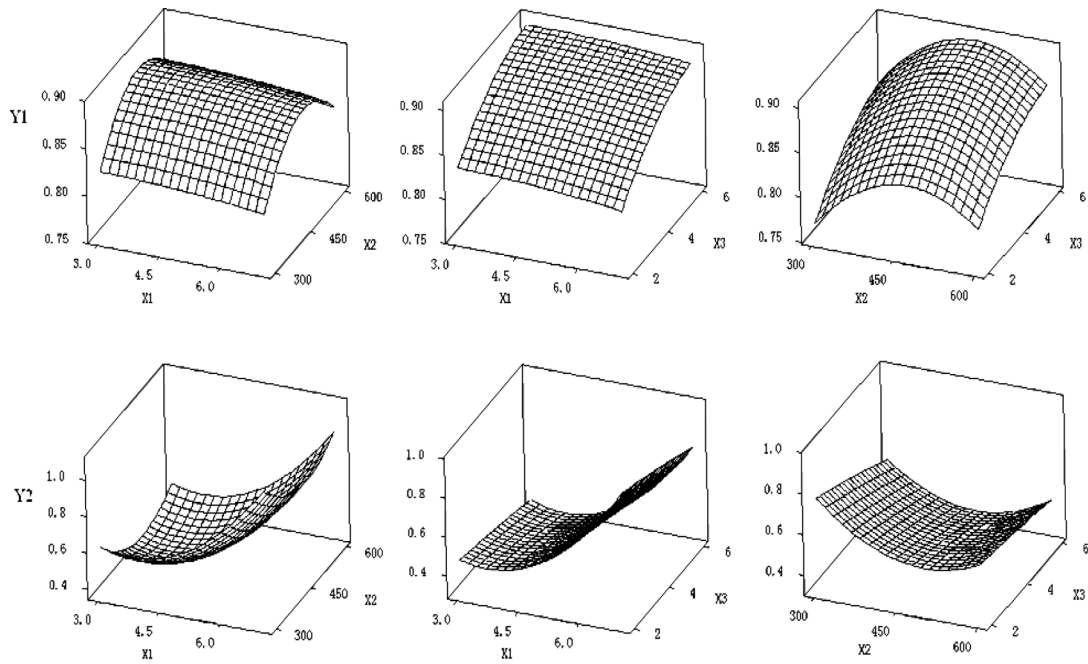


Fig. 1. Effects of Variables ( $X_1$ ,  $X_2$  and  $X_3$ ) on  $Y_1$  and  $Y_2$ : the Response Surface Plots  
 $X_1$ : MCC/lactose (w/w);  $X_2$ : spheronization speed (rpm);  $X_3$ : spheronization time (min);  $Y_1$ : circularity ( $\phi_C$ );  $Y_2$ : friability (%).

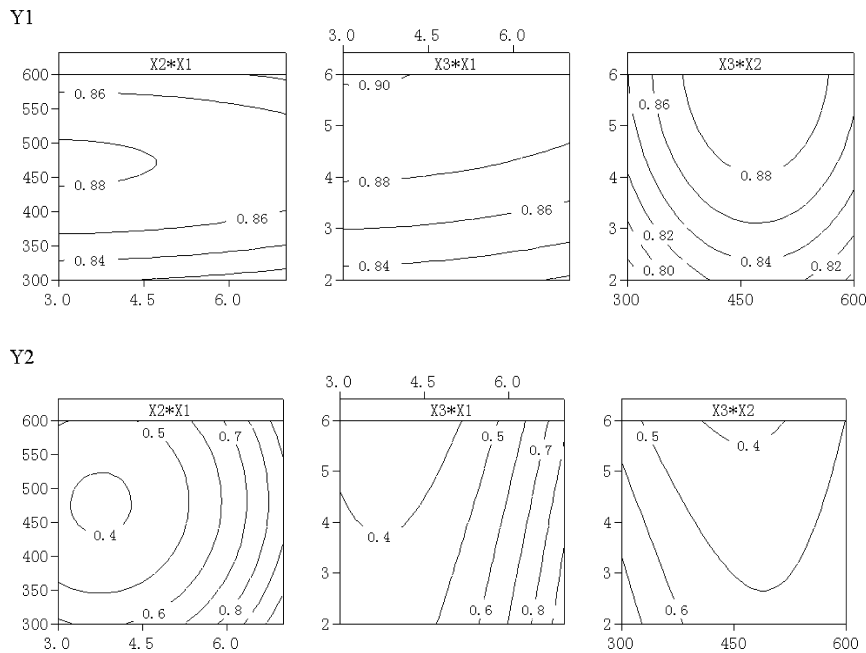


Fig. 2. Effects of Variables ( $X_1$ ,  $X_2$  and  $X_3$ ) on  $Y_1$  and  $Y_2$ : the Contour Plots

spheronization speed ( $X_2$ ) had the major effects on the friability ( $Y_2$ ). Response  $Y_1$  and  $Y_2$  were fitted to linear, two factor interaction and quadratic model respectively to find the most fitting equation (Table 3). Both  $Y_1$  and  $Y_2$  had fitted to the quadratic model with highest correlation coefficients ( $R^2$ ). The equations were:

$$Y_1 = 0.8783 - 0.0060X_1 + 0.013X_2 + 0.036X_3 - 0.0028X_1^2 - 0.047X_2^2 - 0.015X_3^2 + 0.00025X_1X_2 + 0.0013X_1X_3 - 0.00075X_2X_3, \\ R^2 = 0.9068$$

$$Y_2 = 0.4627 + 0.23X_1 - 0.056X_2 - 0.075X_3 + 0.19X_1^2 + 0.15X_2^2 - 0.0068X_3^2 - 0.0058X_1X_2 - 0.018X_1X_3 - 0.031X_2X_3,$$

$$R^2 = 0.9421$$

MCC/lactose ratio ( $X_1$ ) had major effect on  $Y_2$ , while  $Y_1$  was less influenced. A lower MCC/lactose ratio would reduce the friability, because lactose could generate an inner capillary force which maintained the moisture to reinforce the binding of the mass and to reduce the adhesion among pellets. Adjusting of spheronization speed ( $X_2$ ) could slightly depress the effect of  $X_1$  on the product's friability. Spheronization speed ( $X_2$ ) had strong effects on both  $Y_1$  and  $Y_2$ . A lower spheronization speed could not provide enough energy for the plastic deformation of wet mass, so the particles

Table 3. Correlation Coefficients of Fitting Responses to Various Models

Model	$R^2$	
	$Y_1$	$Y_2$
Linear	0.4366	0.6151
Interaction	0.2260	0.4839
Quadratic	0.9068	0.9421

Table 4. Optimized Variables and the Corresponding Responds by Box–Behnken Experimental Design

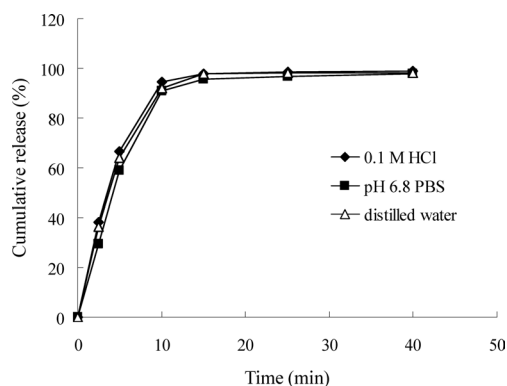
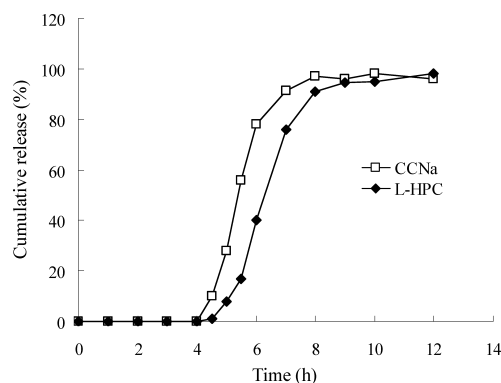
Factor	$X_1$	$X_2$	$X_3$
Optimized	3.85	460.6	6.0
Response	$Y_1$	$Y_2$	
Weight	1.0	1.0	
Target	0.900	0.200	
Desirability	1.0000	0.80076	
Predicted	0.901	0.320	
Observed	0.890	0.332	

could hardly be spheronized. On the other hand, at higher spheronization speeds, the increased friction within the particles would accelerate the moisture evaporation and form a rigid shell on the particle surface, which would reduce the plasticity of the pellets and lead to insufficient spheronization. Spheronization time ( $X_3$ ) was another important operating factor. It influences not only the shape of the pellets but also the compactness, which will affect the drug release rate of the final product. Therefore, the spheronization time was limited to be less than 6 min.

When simultaneously meeting the standard of maximized  $Y_1$  and minimized  $Y_2$  with the best desirability, the optimized variables were shown in Table 4. According to the optimized conditions, three batches of pellets were prepared and evaluated. The observed responses had good concordance with the predicted ones (Table 4).

**Drug Release from Uncoated Cores** The drug release rate was another important concern for the pellets. In the case of pulsatile delivery system for ISMN, when lag time was passed, the drug should be released steadily and sufficiently within a short period of time. The release rate was affected by the composition of the formulation and the operating conditions. The release pattern of the uncoated pellets from the optimized formulation in various media is shown in Fig. 3. In each of the dissolution media, more than 90% of the drug content released within 10 min. Comparing to the coated ones, the drug release was rapid and sufficient. Therefore, the release from the cores was not the rate-limiting step of the overall drug release.

**Study on Swelling Layer** Swelling layer prevents the drug containing cores to contact directly with the aqueous media; on the other hand, after swelling by hydration it ruptures the outer coating to release the drug. The swelling characters of this layer are responsible for the formation of lag time and the subsequent drug release. In this study, L-HPC and CCNa, respectively, were evaluated as swelling layer, both at a level (weight gain) of 24%. The outer control layer was Surelease<sup>®</sup> (aqueous dispersion of ethylcellulose) at a

Fig. 3. Drug Release from Uncoated Pellet Cores in Various Media  $n=6$ .Fig. 4. Drug Release of ISMN-PRP with Different Type of Swelling Layer The coating level was 20% (w/w),  $n=6$ .

weight gain level of 16%. Drug release profiles are as shown in Fig. 4. At the experimental level, CCNa had a shorter lag time than that of L-HPC, about 1 h shorter. The rate and quantity of drug release were similar. There was no major difference between the two polymers evaluated as swelling layer for the pulsatile drug release. However, the high viscosity of L-HPC solution led to blockage of the spray nozzle in the coating process. A lower L-HPC concentration could avoid the blockage, but would prolong the coating time which increased the aggregation of pellets. Therefore, CCNa was chosen as swelling layer when considering the operating convenience.

Effect of the thickness of swelling layer was evaluated. In this study, the layer thickness was expressed as the weight gain (%) of the coating. Different levels of swelling layer (0%, 16%, 20%, 24%, 28%) were coated and investigated on the drug release pattern, as is shown in Figs. 5 and 6. In the case of without swelling layer (0%), the drug release started with a slow rate and a lag time of 7.0 h, and at 12 h only 25% of the total drug was released. In the absence of swelling layer, the ethylcellulose membranes kept integrity throughout the whole process. Water permeated into the drug containing cores and dissolved the drug. When a drug concentration gradient was formed, the drug permeated through the membranes at a relatively slow speed. The permeation and dissolution processes led to the prolonged lag time. When 16% level of CCNa was coated, the lag time was 5.1 h and the release time was 1.4 h. When the coating level increased to 20% or higher, the lag time shortened to about 4.2 h. The in-

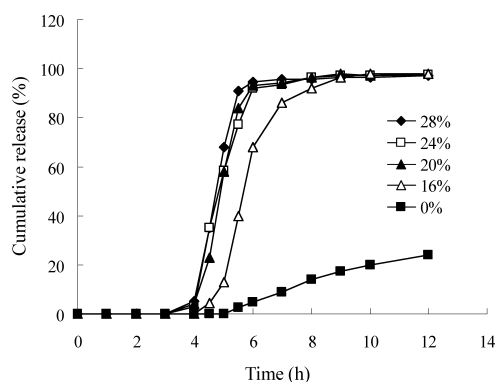


Fig. 5. Drug Release of ISMN-PRP with Different Levels of CCNa as Swelling Layer Coating

*n*=6.

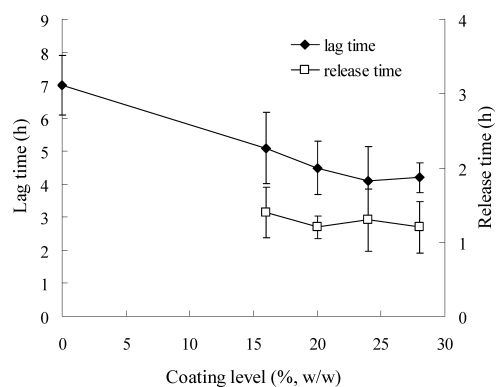


Fig. 6. Lag Time and Release Time of ISMN-PRP with Different Levels of CCNa as Swelling Layer Coating

Error-bars represent S.D., *n*=6. The release time of 0% (w/w) of CCNa was not detected.

crease in coating level up to 28% did not show significant difference with that of 20% in term of lag time or release time. Therefore, 20% of weight gain was acceptable for the swelling layer.

**Study on Control Layer** Surelease<sup>®</sup>, the commercially available ethylcellulose aqueous dispersion was chosen as the outer membrane. Surelease<sup>®</sup> which has medium chain triglycerides as plasticizer was employed directly for coating, and the drug release is independent to the circumstance pH value. Different coating levels of control layer (12%, 14%, 16%, 18%, 20%) were investigated while the swelling layer was set at 20%, as is shown in Figs. 7 and 8. The lag time was 2.7, 3.6, 4, 4.9 and 5.8h when the coating level was 12%, 14%, 16%, 18% and 20%, respectively. The control layering level had a significant influence on the lag time, because the swelling energy required for rupture increased with the thickness of control layer. At the fixed level of swelling layer, when the control layer was thickened, a longer time was needed to absorb enough water to get the critical swelling pressure. On the other hand, the drug release rate was not significantly influence by the thickness of control layer. The expecting lag time for pellets containing ISMN was 4 h, and therefore a control layer of 16% and a swelling layer of 20% were chosen to obtain an ideal lag time.

**Effect of Size of the Cores** Pellet cores with different diameters were prepared by adjusting the size of the screen of extruder. The effect of core size is shown in Fig. 9. The

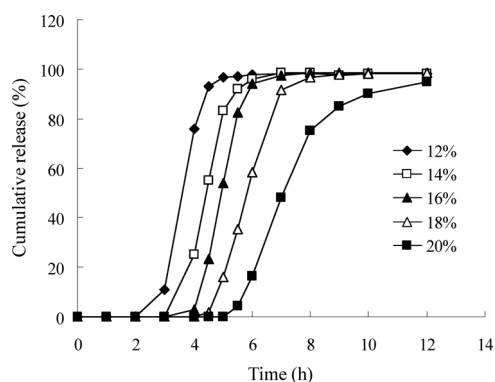


Fig. 7. Drug Release of ISMN-PRP with Different Levels of Control Layer Coating

The swelling layer was 20% (w/w) CCNa, *n*=6.

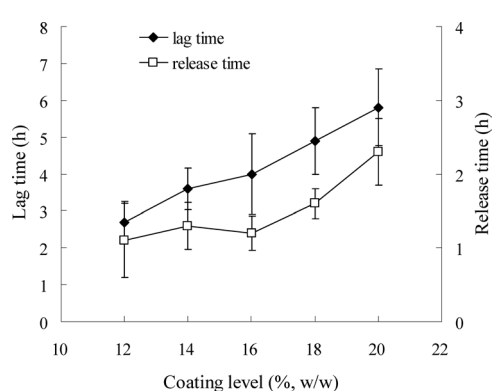


Fig. 8. Lag Time and Release Time of ISMN-PRP with Different Levels of Control Layer Coating

Error-bars represent S.D., *n*=6.

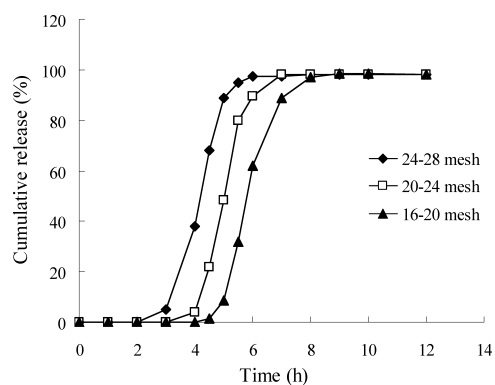


Fig. 9. Effect of Size of Core Pellets on the Drug Release Pattern of ISMN-PRP

*n*=6.

coating level of swelling layer and control layer were 20% and 23%, respectively. The lag time was significantly influenced by the core size, while the release rate was only slightly influenced. When the particle size was reduced, the lag time was shortened accordingly. This is because that the specific surface area increased when the pellets were smaller, and led to a thinner layer when the coating weight gain was constant. And as was discussed above, the lag time decreased as the thickness of controlling layer falling.

**Influence of Dissolution Media** The drug release pat-

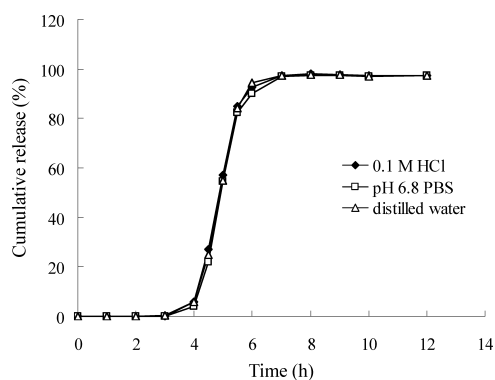


Fig. 10. ISMN Release Form Pulsatile Release Pellets in Various Media  $n=6$ .

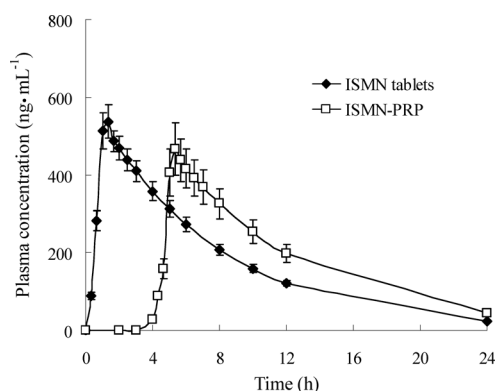


Fig. 11. Plasma Concentration vs. Time Profile of ISMN after Oral Administration in Beagle Dogs

Test: ISMN-PRP; reference: ISMN tablets. Error-bars represent S.D.,  $n=6$ .

tern in distilled water, 0.1 M HCl or PBS pH 6.8, respectively, was investigated. As is shown in Fig. 10, the drug release patterns in various media did not show significant differences. The drug release behavior was not influenced by the variety of dissolution media.

**Pharmacokinetics in Beagle Dogs** The pharmacokinetics of ISMN pulsatile release pellets (ISMN-PRP) after oral administration to beagle dogs was studied, while using commercially available ISMN tablets as the reference formulation. The ISMN concentrations versus time profiles are as shown in Fig. 11. The corresponding pharmacokinetic parameters are presented in Table 5. It was found that the lag time of ISMN-PRP after orally taken by beagle dogs was 4.094 h, comparing to 0.25 h of the reference tablets. In the *in vitro* dissolution test, the lag time of ISMN-PRP was 4.1 h, which indicated that the *in vivo* pulsatile release pattern had good correlation with that of *in vitro*. This could be attributed to the pH-independent release profile of the ISMN-PRP. The  $C_{\max}$  of ISMN-PRP was slightly lower than that of ISMN

Table 5. Pharmacokinetics Parameters in Beagle Dogs after Oral Administration,  $n=6$ , mean  $\pm$  S.D.

Parameters	ISMN tablets	ISMN-PRP
$T_{\text{lag}}$ (h)	0.250 $\pm$ 0.110	4.094 $\pm$ 0.21
$T_{1/2}$ (h)	5.036 $\pm$ 0.147	5.155 $\pm$ 0.352
$C_{\max}$ (ng $\cdot$ mL $^{-1}$ )	552.086 $\pm$ 69.075	479.216 $\pm$ 77.352
$T_{\max}$ (h)	1.165 $\pm$ 0.311	5.277 $\pm$ 0.520
$AUC_{(0-t)}$ (h $\cdot$ ng $\cdot$ mL $^{-1}$ )	4185.622 $\pm$ 345.103	3994.378 $\pm$ 416.329
$AUC_{(0-\infty)}$ (h $\cdot$ ng $\cdot$ mL $^{-1}$ )	4373.492 $\pm$ 403.680	4392.542 $\pm$ 448.192
$K_e$ (h $^{-1}$ )	0.138 $\pm$ 0.091	0.135 $\pm$ 0.086

tablets, and this might be due to that after the lag time, the drug absorption happened in the posterior section of small intestine where the absorption rate was relatively slow. By comparing the area under the time-concentration curve ( $AUC$ ) $_{(0-\infty)}$ , ISMN-PRP had an equivalent bioavailability with the reference formulation. The *in vivo* data demonstrated that ISMN-PRP could perform a pulsatile drug release at predetermined time point without loss of bioavailability. Its pharmacokinetics and pharmacodynamics in human will be further studied.

## Conclusion

The three-layered ISMN pellets which were coated with CCNa as swelling layer and ethylcellulose aqueous dispersion Surelease<sup>®</sup> as outer control layer achieved a pulsatile release of ISMN with 4.1 h of lag time, both *in vitro* and *in vivo*. The formulation of the pellet cores and its extrusion-spheronization process was optimized by Box-Behnken experimental design, and the circularity and friability of the optimized product had a good concordance with the predicted values from the model. The lag time and the release time of ISMN-PRP were influenced by the coating levels of swelling layer and control layer, which were recognized as the composition of 20% (weight gain, w/w) of CCNa and 16% of Surelease<sup>®</sup>. Pharmacokinetics in beagle dogs demonstrated that the *in vitro* pulsatile release was also valid *in vivo*.

## References

- 1) Haus E., *Adv. Drug Deliv. Rev.*, **59**, 985–1014 (2007).
- 2) Portaluppi F., Lemmer B., *Adv. Drug Deliv. Rev.*, **59**, 952–965 (2007).
- 3) LaBounty T., Eagle K. A., Manfredini R., Fang J., Tsai T., Smith D., Rubenfire M., *Clin. Cardiol.*, **29**, 542–546 (2006).
- 4) El-Maradny H. A., *Drug Deliv.*, **14**, 539–546 (2007).
- 5) Mohamad A., Dashevsky A., *Int. J. Pharm.*, **318**, 124–131 (2006).
- 6) Maeda H., Ogawa Y., Ishiyama M., Hirayama T., Terada K., *Chem. Pharm. Bull.*, **56**, 464–467 (2008).
- 7) Paterakis P. G., Korakianiti E. S., Dallas P. P., Rekkas D. M., *Int. J. Pharm.*, **248**, 51–60 (2002).
- 8) Chopra S., Motwani S. K., Iqbal Z., Talegaonkar S., Ahmad F. J., Khar R. K., *Eur. J. Pharm. Biopharm.*, **67**, 120–131 (2007).
- 9) Pastera J., Vyslouzil L., Kvetina J., *J. Chromatogr. B, Analyt. Technol. Biomed. Life Sci.*, **800**, 271–274 (2004).