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Evaluation of novel immediate-/controlled-release tablets of isosorbide-5-mononitrate (5-ISMN): *in vitro-in vivo* correlation

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The aim of the present study was to develop the novel immediate-controlled release (ICR) tablets of isosorbide-5-mononitrate (5-ISMN) composed of an osmotic pump tablet core coated with an immediate-release layer. The novel ICR tablets of 5-ISMN could release drug quickly and continuously through a semi-permeable membrane (SPM) composed of ethylcellulose (EC)/polyethylene glycol (PEG) 4000 and cellulose acetate (CA)/PEG4000. Release tended to decrease with storage time. However, the drug release rates changed little for the SPM composed of EC/PVP K30. The weight loss test also confirmed these results. The major release mechanism was diffusion according to the Higuchi equation. The relative bioavailability of the ICR tablets compared to the reference formulation in the single and multiple dose regiments were 90.9 and 111.2%, respectively. They were both bioequivalent to the reference formulation. *In vitro-in vivo* correlation (IVIVC) studies demonstrated that the dissolution *in vitro* simulated the absorption *in vivo* well. In general, 5-ISMN ICR tablets composed of an osmotic pump tablet core and an immediate-release layer may be promising in providing immediate and constant drug delivery with minimum fluctuations during long storage time.

1. Introduction

The indications of isosorbide-5-mononitrate (5-ISMN) include long time treatment of coronary heart disease, prevention of angina, continuous treatment of angina after myocardial infarction (Fotaki et al. 2005; Parker and Parker 1998). The earliest common formulation of 5-ISMN was required to be taken 2 or 3 times every day (Bonn 1988). This might cause great fluctuations of plasma concentration, high C_{max} and severe side effects. Sustained-release tablets of 5-ISMN developed later were taken once daily (Thomas et al. 2007; Walker et al. 1996), and decreased C_{max} and side effects to some extent. However, the drug needed time to release and was not appropriate for the emergency treatment. Novel 5-ISMN formulations combined the immediate and controlled release (ICR) segments in order to exert the immediate and prolonged effect, such as ICR pellets in capsules produced by Schwarz Pharma Co., Ltd. (Elantan® 50 mg, Germany), Huayu Pharmaceutical Factory (Zaisheng® 50 mg, Wuxi, China), and SaikePharma Co., Ltd. (Aisimo® 40 mg, Beijing, China). It might help relieve the angina symptom quickly, simultaneously keep the effective plasma concentration for a longer time, and decrease the side effects.

However, the release rate of the controlled part in the ICR capsules on the market is not constant. The release and absorption of 5-ISMN is affected by the gastrointestinal fluids *in vivo*. The great fluctuation of plasma concentration is unpredictable and individual differences are obvious. As a novel release system, osmotic pumps could release drugs with a constant rate avoiding aging (Verma et al. 2003). However, release profiles might decrease with time due to the interaction of the semi-permeable membrane (SPM) materials.

ICR tablets are biphasic delivery systems, which are composed of a fast release past and a slow release part. This system is able to produce a rapid rise in the plasma concentration requested to promptly exert the therapeutic effect, followed by an extended release phase in order to avoid repeated administrations (Wei et al. 2006). In addition, biphasic delivery systems are hot research topics (Lopes et al. 2006; Ofori-Kwakye and Fell 2001; Wu et al. 2007).

The major innovations of this study were as follow. First, based on the concept of biphasic delivery, the immediate-release layer was coated outside the controlled-release tablets of ISMN which were prepared previously (Li et al. 2012). The dose of the controlled release tablets was 40 mg. The total 40 mg was divided into 28 mg in the controlled release tablets core and 12 mg in the immediate-relase layer of the biphasic delivery system. Second, the compositions of SPM changed a little due to the different dose and structure of the tablets. Third, the dissolution profiles, fitting equations, and release mechanisms between ICR tablets, CR tablets and sustained-release tablets were compared. Last but not the least, *in vitro – in vivo* correlation (IVIVC) of 5-ISMN ICR tablets were also evaluated. It demonstrated that the release *in vitro* could simulate the absorption *in vivo* well.

2. Investigations, results and discussion

2.1. Morphology of the 5-ISMN ICR tablets

Compared with the osmotic pump tablets of 5-ISMN composed of PEG and PVP, the releasing pore of 5-ISMN ICR tablets were

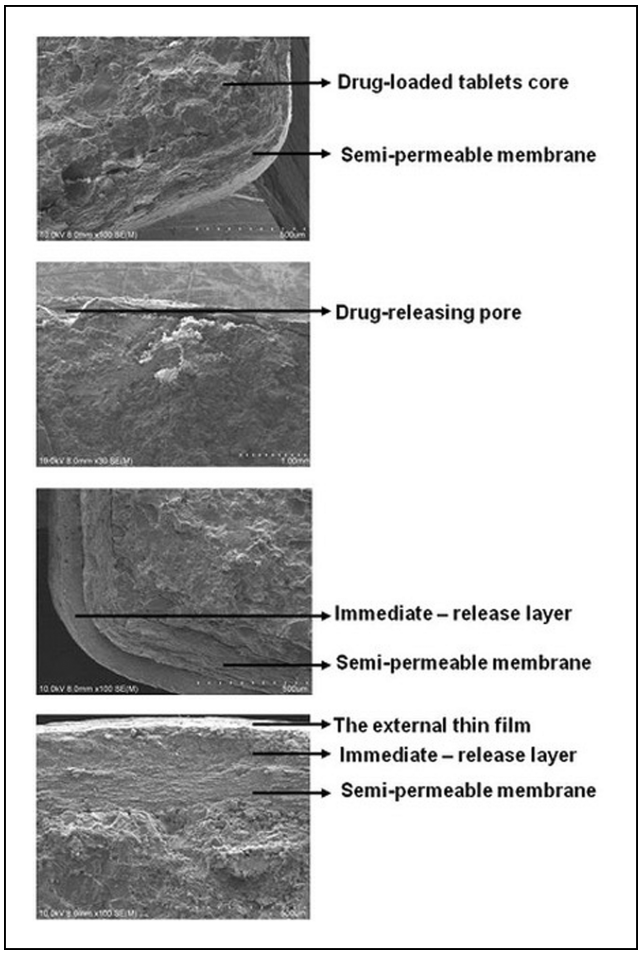

Fig. 1: Morphology of 5-ISMN ICR tablets.

covered by the immediate-release layer and then diminished (Fig. 1). It was proposed that 5-ISMN ICR tablets could release drugs immediately and continuously.

2.2. Drug release and aging tendency of 5-ISMN ICR tablets with EC and PEG as SPM

The initial release profiles of the ICR tablets with EC and PEG as the SPM was steady and in coincidence with the quality standards. However, the release rates decreased with prolonged storage time (Fig. 2 (A)). This corresponded with the results of reduced coating membrane weight. The decreased coating membrane ratio was 38.9% initially, and reduced to 29.7% after 24 months (Fig. 2 (B)). It demonstrated that EC and PEG interacted with each other, resulting in decreased solvable contents and reduced SPM permeability.

2.3. Drug release and aging tendency of 5-ISMN ICT tablets with CA and PEG as SPM

The drug release profiles and aging tendency of the ICR tablets with CA and PEG as the SPM was similar to that of the ICR tablets with EC and PEG as the SPM (Fig. 2 (C)). The drug release rates decreased significantly after storing for longer time. The cumulative release at 7 h was 96.7% (0 month) initially and was reduced to 80.4% after 24 months (Fig. 2 (C)).

The time-dependent results of decreased coating membrane were similar. At 1 h, the membrane loss ratio of the ICR tablets at month 0 was 32.4% and decreased to 20.1% after storing for 24 months. At 2 h, the membrane loss ratio of the ICR tablets at month 0 was 32.6% and decreased to 20.1% after storing for 24 months. The continuous interaction of CA and PEG generated more hardly solvable segments and decreased the permeability of the SPM (Fig. 2 (D)).

The components of SPM were very important for the drug release profiles. The soluble plasticizer – PEG dissolved in water and formed micropores on the SPM facilitating the drug release process. However, during storage PEG tended to cross-link with EC, leading to a decrease of the dissolved segments. As a result, the drug release rate decreased during the storage period. In addition, due to the decreased permeability caused by the membrane polymers crosslinking, water could hardly enter the tablets core through the surface. Therefore, drugs did not dissolve to generate a saturation solution and could hardly be released from the core, resulting in decreased release profiles. With prolonged storage time, the decreasing drug release tendency might because more apparent and increased drug residues, and non-complete release can be expected (Fig. 2).

2.4. Constant release profiles and aging tendency of the novel ICR tablets with the SPMs composed of EC and PVP

The release profiles of 5-ISMN ICR tablets with the SPM composed of EC and PVP were constant compared to that composed of EC/PEG and CA/PEG. As 5-ISMN ICR tablets with the SPM weight increasing ratio of 6% storing for 0, 6, 12, 24 months, the cumulative release at 0.5, 1, 4, 7 h were 30.9 ± 0.3, 44.6 ± 0.4, 76.5 ± 0.3, 98.5 ± 0.4, respectively. The cumulative release of 5-ISMN ICR tablets with the SPM weight increasing ratio of 8% at 0.5, 1, 4, 7 h were 29.9 ± 0.1, 39.3 ± 0.3, 70.3 ± 0.4, 98.5 ± 0.3, respectively. As the SPM increasing ratio of 10 and 12%, the cumulative release at different time points were 32.0 ± 0.2, 41.6 ± 0.3, 72.2 ± 0.2, 99.2 ± 0.2 and 31.8 ± 0.3, 42.6 ± 0.4, 75.5 ± 0.4, 98.5 ± 0.2, respectively (Fig. 3). The little standard deviation of the membranes loss results implied that the release profiles were independent of the storage time and the aging effect was minimum. One possible reason could be that EC and PVP kept the initial ratio and did not crosslink with each other during storage.

The combination of EC and PVP was the most common membrane material for sustained release micropellets. The most important characteristic was that the membrane was not semi-permeable, meaning it could imbibe water and release drug at the same time. However, drug release from osmotic pump tablets is based on the principles of osmotic pressure (Marucci et al. 2010). The mechanism is that the membrane permits water to enter the core and drugs are released from the predetermined micropore in the membrane. This release pattern fits zero-order release profiles. Utilization of EC as SPM containing PVP as plasticizer was a new approach to prepare an osmotic pump formulation, taking advantages of EC and PVP not interacting with each other. As a result, this novel approach could avoid aging effect. The novel combination of immediate-release layer on the top of the external membrane can guarantee both immediate and prolonged drug release (Fig. 3).

2.5. Release mechanisms of the different 5-ISMN tablets

The different values of n in the Ritger-Peppas exponent model represent the different release mechanisms. $n < 0.45$ stands for Fick's diffusion and $n > 0.89$ stands for the dissolution controlling mechanism. A value of n between 0.45 and 0.89 shows that the release mechanism is a combination of Fick's diffusion and dissolution. As for the ICR tablets, $n = 0.421 < 0.45$, implying that Fick's diffusion played an important role. n of the osmotic pump tablets was 1.126, implying that dissolution was the major

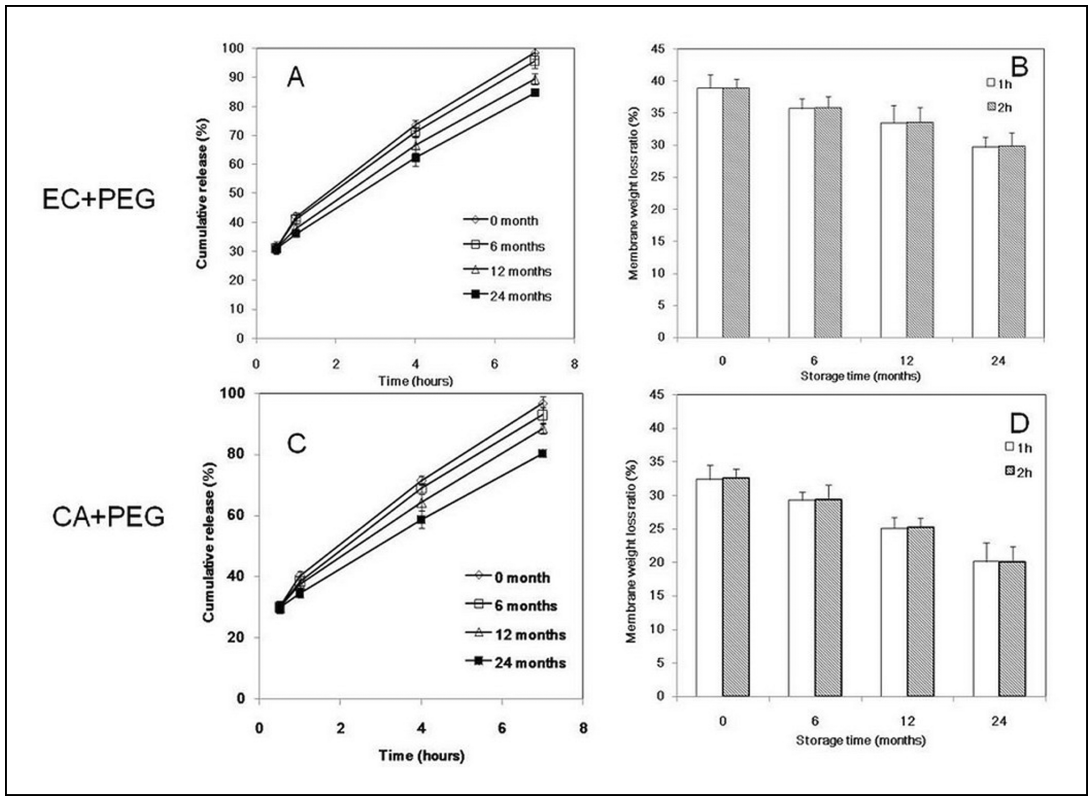

Fig. 2: Cumulative release and membrane weight loss ratio of 5-ISMN ICR tablets with the SPM composed of EC + PEG (A, B) and CA + PEG (C, D), respectively.

mechanism. In addition, if $n > 0.66$, the release fell into the zero order release pattern. As a result, the release of the osmotic pump tablets were zero-order and the drug release rate was constant. The n value of the sustained release tablets was 0.621, implying that the release mechanisms were a combination of diffusion and dissolution. The drug release rate was not constant and not appropriate for a steady and efficient drug plasma concentration (Table 1).

The different fitting models of 5-ISMN ICR tablets, CR tablets and the marketed sustained-release tablets are summarized in Table 1. The Higuchi equation were fitted well for all of the three formulations, impling that diffusion mechanism played an important role in the drug release (Baidya et al. 1999). Based on the results, it is proposed that an appropriate diffusion coefficient of the membrane was of great significance for drugs release (Lin 1993).

2.6. Bioavailability and IVIVC evaluation

5-ISMN plasma concentration profiles of the single and multiple dose experiments of the reference formulation and 5-ISMN ICR tablets are shown in Fig. 4. As for the single dose regiment, there were no significant differences of the C_{max} (bilateral one-side test) and T_{max} (Wilcoxon rank sum test) values observed between the two formulations. The relative bioavailability of the ICR tablets against the reference formulation was $90.9 \pm 16.3\%$. $AUC_{0\text{-}t}$ and C_{max} were 111.3%, 123.3%. This meant that the ICR tablets with EC and PVP as the plasticizer were pretty much bio equivalent to the reference formulation (Table 2).

In the multiple dose regimen there were no significant difference of the C_{max} (bilateral one-side test) and T_{max} (Wilcoxon rank sum test) values observed between Xinkang^{TM} sustained release tablets and 5–15MN ICR tablets (Table 3). The relative bioavailability of the ICR tablets against the reference formulation was $111.2 \pm 24.3\%$. $AUC_{0\text{-}t}$ and C_{max} were 116.6%, 120.4% consistent with the results aforementioned. In general, the 5-ISMN ICR tablets were bioequivalent to the marketed sustained-release tablets with more constant, plasma concentration which confirmed that the ICR tablets can be more efficient in terms of both immediate and continuous drug release (Table 3).

Evaluation methods that more closely imitate the *in vivo* situations are less appropriate for mass screening of the formulations as they are more labor-intensive and material-consuming. The common basket/paddle method is approved for investigation of drug release and solubility. The question was, however, whether this method could represent the drug absorption *in vivo*. To that

Table 1: Release kinetic parameters and correlation coefficients of different equations for 5-ISMN ICR tablets, osmotic pump tablets, and the sustained-release tablets

	ICR tablets		CR tablets		Sustained-release tablets	
	Equation	R	Equation	R	Equation	R
Zero-order	$F_t = 10.11t + 29.61$	0.9965	$F_t = 15.87t - 0.336$	0.9945	$F_t = 11.75t + 18.75$	0.9965
First-order	$Ln(1-F_t) = 0.641t + 4.866$	0.9402	$Ln(1-F_t) = 0.515t + 5.147$	0.9839	$Ln(1-F_t) = 0.365t + 4.717$	0.9905
Higuchi	$F_t = 34.00t^{0.5} + 7.231$	0.9975	$F_t = 55.39t^{0.5} - 43.10$	0.9995	$F_t = 40.90t^{0.5} - 12.71$	0.9990
Ritger-Peppas	$LnF_t = 0.421lnt + 3.739$	0.9975	$LnF_t = 1.126lnt + 2.589$	0.9940	$LnF_t = 0.621lnt + 3.361$	0.9995
Hixson-Crowell	$F_t^{1/3} = 0.216t + 3.181$	0.9859	$F_t^{1/3} = 0.425t + 2.112$	0.9628	$F_t^{1/3} = -0.270t + 2.885$	0.9829

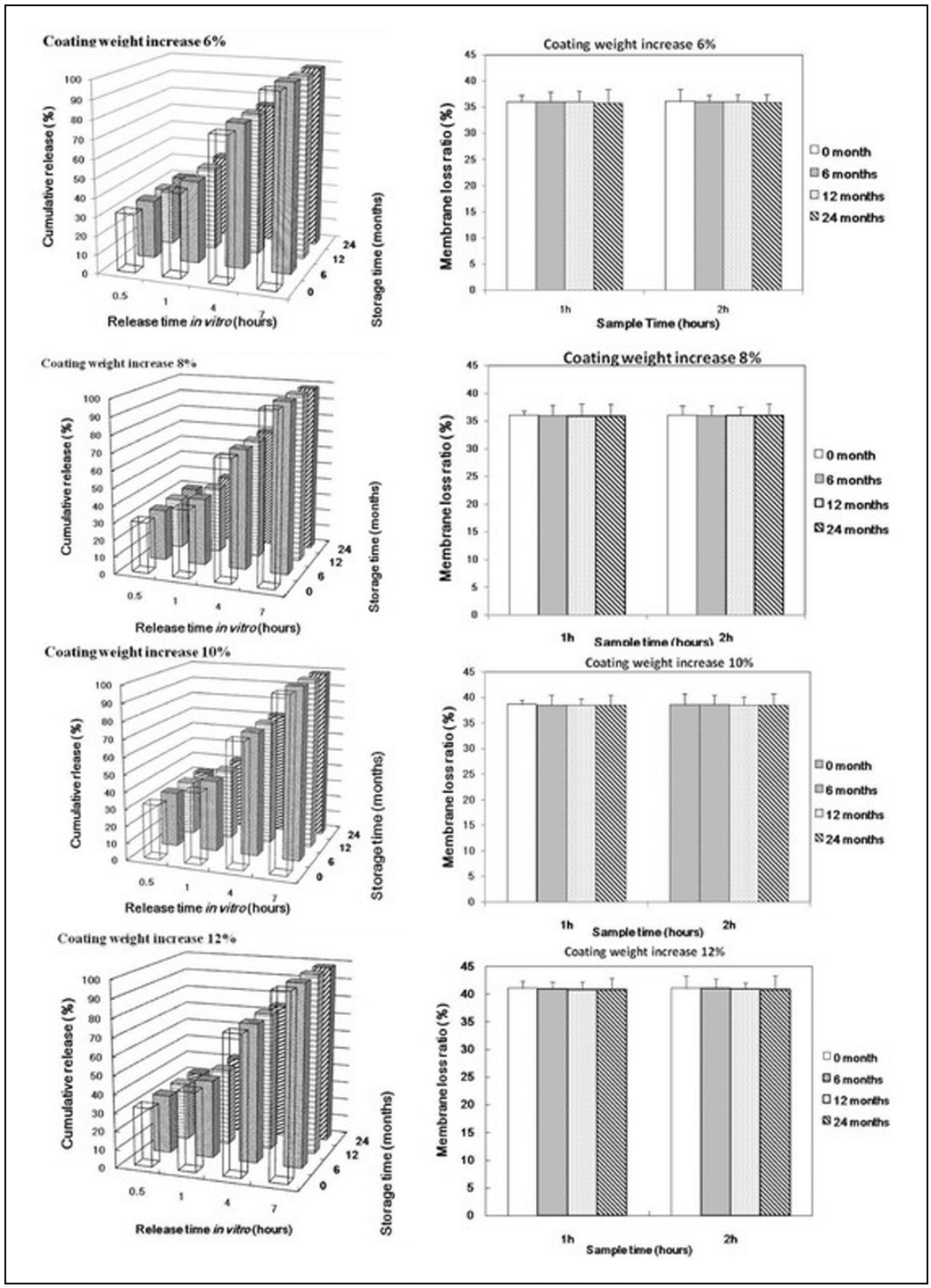

Fig. 3: Constant release profiles and weight loss ratio of the novel 5-ISMN ICR tablets with the SPM composed of EC and PVP.

Table 2: Mean pharmacokinetic parameters for the volunteers following oral administration of the ICR tablets and sustained release tablets of 5-ISMN in the single dose regimen

Parameters	Single dose regimen	
	ICR tablets	Sustained release tablets
C_{max}(ng/ml)	542.90 ± 118.83	551.76 ± 121.63
T_{max} (h)	5.29 ± 1.94	4.60 ± 3.14
$t_{1/2}$ (h)	6.25 ± 0.85	6.25 ± 0.93
$AUC_{(0\text{-}t)}$ (ng/ml*h)	7140.26 ± 1598.3	7961.74 ± 1352.20
$AUC_{(0\text{-}\infty)}$ (ng/ml*h)	7494.52 ± 1577.67	8315.47 ± 1353.45
Relative bioavailability (%)	90.9 ± 16.3	

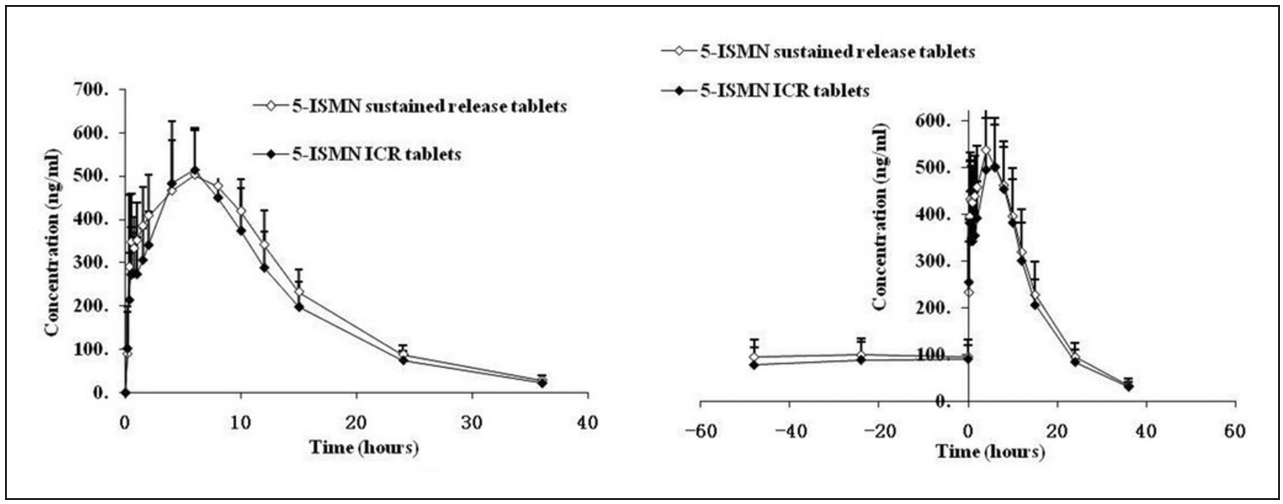

Fig. 4: Pharmacokinetics and IVIVC of 5-ISMN ICR tablets. (A) single-dose pharmacokinetics of 5-ISMN ICR tablets compared with the reference formulation; (B) multiple-dose pharmacokinetics of 5-ISMN ICR tablets compared with the reference formulation.

end, IVIVC evaluation was of great importance. IVIVC helps to establish a predictable relationship between biological processes and the physicochemical properties or characteristics of the same dosage form. It is evaluated if drug release fraction ratio *in vitro* (F_r) represented accurately its absorption fraction ratio (F_a) *in vivo*. Level A represents a point-to-point relationship between *in vitro* dissolution and *in vivo* input rate of the drug, as described in the China Pharmacopoeia Appendix XIX D.

The Biopharmaceutical Classification System (BCS) is used to classify drug substances based on their aqueous solubility and absorption *in vivo* (Amidon et al. 1995). The FDA drug evaluation and research center has reported that 5-ISMN is a drug of "high solubility". In addition, 5-ISMN is absorbed totally after oral administration with no first-pass metabolism (Abshagen et al. 1981; Kramer 1994). Therefore, 5-ISMN can be classified to BCS class I based on its high solubility and absorption *in vivo*. Drug release *in vitro* of 5-ISMN might predict its absorption *in vivo* to some extent.

Level A IVIVC was established with the correlation coefficient (r) of 0.9823 for 5-ISMN ICR tablets and the linear regression equation was $F_a = 0.634F_r + 0.534$. $r > r_{4,\ 0.001}$ ($r_{4,\ 0.001} = 0.974$), indicating well fitted IVIVC results. The correlation coefficient (r) is a criterion to evaluate the fitness of the linear regression equation with the data. The closer is r to 1, the better is the correlation between 5-ISMN ICR tablets dissolution and human absorption. The r value (0.974) is good enough to confirm that this *in vitro* model worked very well in simulating *in vivo* conditions.

2.7. Conclusion

In general, the formulated 5-ISMN ICR tablets composed of the osmotic pump tablets core coated with an immediate-release layer is a novel formulation approach that not only can provide immediate treatment but also can afford prolonged release of drugs, thus decreasing plasma concentration fluctuations. Based on the results, diffusion was proposed to be the major release mechanism. IVIVC demonstrated dissolution *in vitro* could imitate the absorption *in vivo* well. Due to these unique features, this novel approach should be of great promise in providing immediate and constant drug delivery with minimum fluctuations in emergency treatment.

3. Experimental

3.1. Materials

5-ISMN was purchased from Lunan Pharmaceutical Group (Shandong, China). PVP K30 and PEG 4000 were obtained from International Specialty Products, Inc. (New Jersey, U.S.A.) and Dow Chemical Company (Beijing, China), respectively. CA was from Eastman Chemical Company (Tennessee, U.S.A.). Sucrose and lactose were from Sifang Industry Co., Ltd. (Xinjiang, China). Magnesium stearate, silicon dioxide and hydroxypropyl methylcellulose (HPMC) E5 was from Huzhou Zhanwang Pharmaceutical Company (Jiangsu, China). Ethylcellulose N100 was from Ruitai Chemical Industry Co., Ltd. (Shandong, China). The sustained release tablets of 5-ISMN as the reference product in the bioavailability test was Xinkang® (40 mg) from Lunan Pharmaceutical Group (Shandong, China). Nitroglycerin as the internal standard was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). All of the other chemicals used were of analytical grade.

The bioavailability experiments were carried out in the First Affiliated Hospital of Lanzhou University and approved by the Ethics Review Board of Lanzhou University.

3.2. Preparation of 5-ISMN ICR tablets

The preparation procedures of the novel 5-ISMN ICR tablets included the preparation of the tablets core, SPM coating, heat treatment at 40 °C, pole

Table 3: Mean pharmacokinetic parameters for the volunteers following oral administration of the ICR tablets and sustained release tablets of 5-ISMN in the multiple dose regimen

Parameters	Multiple dose regimen	
	ICR tablets	Sustained release tablets
C_{max}(ng/ml)	574.28 ± 82.83	554.75 ± 88.18
T_{max} (h)	4.12 ± 2.30	4.67 ± 2.41
$t_{1/2}$ (h)	6.76 ± 0.74	6.74 ± 1.05
C_{av}(ng/ml)	317.54 ± 58.92	295.56 ± 50.36
AUC_{ss}(ng/ml*h)	7620.91 ± 1414.07	7093.31 ± 1208.71
Relative bioavailability (%)	111.2 ± 24.3	

formation with laser, isolation layer coating, and immediate - release layer coating, and the film coating on the external surface (Fig. 1). The preparation of the drug-loaded tablets core was based on the procedures described previously (Li et al. 2012). In brief, 28 g 5-ISMN and 100 g sucrose were sieved with 60 mesh sieve and mixed with 120 g lactose in a wet type granulator (SMG2-6, Chongqing Enger Granulating & Coating Technology Co., Ltd., Chongqing, China) for 10 min. Subsequently, 8% (w/v) PVP K30 ethanol aqueous solution (70%, v/v) were added in the mixture followed by wet granulation. The obtained granules were then dried at 40 °C and screened through a sieve with a pore size of 850 μm. Magnesium stearate (3 g) and 1 g silicon dioxide were mixed with the granules and the resulting mixture was compressed into tablets using a rotary tabletting machine (ZPW23, Shanghai Tianxiang&Chentai Pharmaceutical Machinery Co., Ltd., Shanghai, China) with 9-mm diameter punches. The tablets hardness was maintained within a range of 5 - 7 $kg \cdot cm^{-2}$.

The obtained tablets were coated with SPM using different coating solutions to prepare the osmotic pump tablets for controlled release (CR). Coating was conducted by a highly efficient coating machine (BG1-5, Beijing Institutes of Aviation Manufacturing Technology, Beijing, China) under the following coating conditions: inlet pressure ($3\ kg \cdot cm^{-2}$), spray pressure ($1\ kg \cdot cm^{-2}$), temperature (40 ± 2 °C), and a spray rate of 13 ± 1 ml min^{-1}. The SPM composed of EC and PVP were with the weight increase ratios of 6, 8, 10, 12%, respectively. The coated tablets were treated at 40 °C for 12 h to remove the residual solvent and promote the cross-linking of the coating membrane. Then a 0.6 mm orifice for drug release was formed by the laser punching machine (RC-YW-30, Nanjing Ruichi Electronic Technology Co., Ltd., Nanjing, China) on one side of the tablets (Fig. 1).

The isolating coating solution containing Obady II (10%, w/v) was sprayed onto the obtained tablets with the coating machine. Coating process was continued until a weight gain of 3 % (w/w) was achieved.

The immediate-released membrane coating process was conducted as follows. 50 g HPMC E5 and 30 g 5-ISMN were dissolved in 400 ml ethanol and 600 ml water. The tablets coated with the isolated membrane aforementioned were coated with the immediate-released membrane again. The coating weight gain was 32 mg and 5-ISMN dose was 12 mg.

The same coating solution containing Obady II (10%, w/v) mentioned above was sprayed onto the obtained tablets utilizing a highly efficient coating machine. The coating on the external surface was continued until a weight gain of 3 % (w/w) was achieved (Fig. 1).

The major differences of the ICR tablets prepared in this paper and the CR tablets prepared previously were the different formulation components of the tablets core (Table 4).

The morphology of the final 5-ISMN ICR tablets were examined by scanning electric microscopy (HITACHI S4800, Japan) after painted with platinum (Fig. 1).

3.3. Determination of 5-ISMN by HPLC

5-ISMN contents of the release samples at different sample points were determined by a L-2130 Hitachi high-performance liquid chromatography (HPLC) system (Japan), consisting of L-2130 pump, L-2400 UV detector, and Hitachi D-2000 chromatographic workstation software. A Diamonsil C_{18}-ODS column (4.5 mm × 150 mm, 5 μm) was used for separation. 210 nm wavelength was chosen to measure 5-ISMN. The mobile phase consisted of methanol and water (25:75, v/v) and the flow rate was 1.0 ml · min^{-1}.

3.4. Dissolution in vitro and stability study

In vitro release of 5-ISMN from the tablets were studied with the paddle type apparatus (Tianjin University Radio Factory, Tianjin, China) with 500 ml deionized water at 37 ± 0.5 °C, and the stirring rate was 50 rpm. 5 ml solution was withdrawn and the fresh medium of the same volume was replenished at 0.5, 1, 4 and 7 h, respectively. The obtained samples were filtered (0.45 μm) immediately and analyzed by HPLC with the injection volume of 20 μL (Li et al. 2012, 2011). All experiments were repeated for three times. Stability study was conducted at 25 ± 2 °C with the relative humidity 60 ± 10% for 24 months. Dissolution profiles of the samples at 0, 6, 12, 24 months were performed with the method described above.

The release profiles *in vitro* were described by some models, including zero order, first order, Higuchi equation, Ritger-Peppas exponent equation and Hixson-Crowell equation. The equation fitting was calculated with Excel 2003 Software (Microsoft Corporation, Washington, USA) and the best model was that with the highest correlation coefficient. The drug release mechanisms could be deduced based on the well known equations, possibly involving diffusion and degradation, or the mixed mechanisms. Equations were as follows:

3.5. Weight loss evaluation of the SPM

The weight loss test of SPM could be used to simulate the membrane aging. If continuous interactions occur to the polymers in the SPM during storage time, the ratio of soluble components should decrease, thus leading to a decreased weight loss. On the other hand, the weight loss will stay roughly the same if the membrane polymers do not crosslink.

The following procedures were applied to obtain accurate weights of the SPM and the weight loss ratio was calculated. The thin film, which accounted for immediate-released layer and isolating membrane coating outside the 5-ISMN tablets, were removed and SPM was peeled off carefully. After carefully brushed to remove the residual powder, the SPM was weighed (W_0). The obtained membrane were then added into 500 ml deionized water at 37 ± 0.5 °C controlled by a basket type apparatus (Tianjin University Radio Factory, Tianjin, China) with the stirring rate 50 rpm. SPM were taken out of water after 1, and 2 h stirring, respectively, followed by being dried at 50 °C for half an hour. After cooling to room temperature, the samples were weighed (W_T). Weight loss percent of SPM was calculated according to the following formula:

$$\text{Membrane weight loss}\ (\%) = (1 - W_T/W_0) \times 100\%$$

Dissolution *in vitro* and weight loss tests have been applied to the 5-ISMN ICR tablets that were composed of the different SPM to evaluate the aging tendency. The dissolution *in vitro* and weight loss of the SPM composed of EC and PVP with the weight increase ratios of 6, 8, 10, 12%, respectively were also conducted according to the aforementioned methods.

3.6. Bioavailability studies

3.6.1. Subjects

Healthy male volunteers aged 20 to 30 years with body mass index from 19 to 24 participated in the study. Volunteers were excluded if they were abnormal in the medical examination. All selected volunteers were non-smokers and non-alcoholics and were required to abstain from all drugs and from alcoholic or caffeinated beverages for two weeks before starting and throughout the whole process. The study was approved by the Ethics Review Board of Lanzhou University (China) and conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Ediburgh 2000).

3.6.2. Protocol

The single-dose study design was a fasting and 2-way crossover design. 24 human volunteers were equally divided to two groups randomly. A single dose of the test formulation - 5-ISMN ICR tablets (immediate-release segments of 12 mg and controlled release segments of 28 mg), or the reference formulation - 5-ISMN sustained release tablets (Xinkang®, 40 mg, Lunan Pharma Group, Shandong, China) were administered orally to the volunteers with 200 ml water. Volunteers were required not to take food and drinks for 2 h after dosing and were provided with a standard meal at 4 and 10 h after the drug administration. Blood samples were collected from each volunteer's forearm cubital vein before administration and at 10 min, 20 min, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 15, 24 and 36 h after dosing.The blood samples were centrifuged immediately with $1000 \times g$ at 4 °C and then the plasma was separated and stored at -20 °C until analyzed. The washout period was 2 weeks according to the $t_{1/2}$ of 5-ISMN and then the alternate formulation was given orally.

The multiple-dose experiment was similar to the protocol of the single-dose study except the 24 volunteers were administered orally once daily for consecutive 7 days. Blood samples were collected from each volunteer's forearm cubital vein at different time points, before administration at the fifth and sixth day, 10 min, 20 min, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10, 12, 15, 24 and 36 h after dosing in the seventh day.

3.6.3. Determination of 5-ISMN plasma concentration

Quantification of 5-ISMN in plasma samples were analyzed using a gas chromatograph (GC) with an electron capture detector (ECD) (GC-ECD) which was performed under the following operating conditions. A Shimadzu equipped with a split/splitless injector and a ZB-1 (100 % dimethylpolysiloxane) capillary column (30 m, 0.25 mm (internal diameter), 0.25 μm (film thickness); Agilent Technologies Inc., California, U.S.A.). Nitrogen was as the carrier gas with a flow rate of 2 ml min^{-1}. The column temperature was programmed as initial 140 °C for 6 min, then increased at 50 °C · min^{-1} to 210 °C and ended immediately. Injector and detector temperatures were set at 200 °C and 220 °C, respectively. The split ratio was 12:1. A ChromatoSolution light chromatographic workstation gathered and processed the data throughout the entire experiment (Li et al. 2011; Pastera et al. 2004).

The treatment of blood samples containing 5-ISMN was described as follows. A 200-μl aliquot of plasma sample was mixed thoroughly with the nitroglycerin standard solution in methanol as the internal standard (2 μg ·

Table 4: The differences of the formulation components between ICR and CR tablets (1000 units)

ICR tablets		CR tablets	
Components	Dosage	Components	Dosage
The formulation components of the tablets core		The formulation components of the tablets core	
5-ISMN	28 g	5-ISMN	40 g
Lactose	120 g		
Sucrose	100 g	Sucrose	190 g
8% PVP K30 solution	Appropriate amount	8% PVP K30 solution	Appropriate amount
Magnesium Stearate	3 g	Magnesium Stearate	3 g
Silicon Dioxide	1 g	Silicon Dioxide	1 g
The formulation components of the SPM		The formulation components of the SPM	
EC N100	30 g	EC N100	30 g
PVP K30	19 g	PVP K30	20 g
Ethanol	1000 ml	Ethanol	1000 ml
The formulation components of the isolation layer coating		The formulation components of the isolation layer coating	
Stomach-dissolving doating powder – Obady II	10 g	Stomach-dissolving doating powder – Obady II	10 g
Water	100 ml	Water	100 ml
The formulation components of the immediate-release membrane			
5-ISMN	12 g		
HPMC E5	20 g		
Ethanol	160 ml		
Water	240 ml		

ml^{-1}, 10 µl). Ethyl acetate (1 ml) was added, vortexed for 3 min, and centrifuged at 5000 × *g* for 10 min. The supernatant was pipetted into a clean tube and evaporated to dryness under a gentle stream of nitrogen. The residue was redissolved in 70 µl methanol followed by centrifugating for 5 min at 5000 × *g*. The supernatant was transferred into a tube and 1 µl was injected into GC.

3.6.4. IVIVC

The percentage fraction absorbed (F_a) of 5-ISMN ICT tablets was calculated by the Wagner-Nelson equation as it fitted into the single compartment model (Wang and Nedelman 2002).

$$Fa = \frac{(Ct + k10 \times AUC_{0-t})}{k_{10} \times AUC_{0-\infty}} \times 100\% \quad (1)$$

In Eq. (1), C_t is the concentration at time point *t*, K_{10} is the elimination rate of the dosage form, $AUC_{0\text{-}t}$ is the area under the curve from zero to time *t*, and $AUC_{0\text{-}\infty}$ isthe area under the curve from zero to infinity. The dissolution percentage fraction (F_r) and F_a at the corresponding time points were fitted into a regression equation by the least square method. The regression coefficient was the index of correlation between *in vitro* release and *in vivo* absorption.

3.7. Statistical analysis

The pharmacokinetic parameters were calculated and analyzed by Phoenix WinNonlin version 6.1 (Pharsight Co., Ltd., U.S.A.). $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$ were calculated by the linear trapezoidal rule and extrapolation to infinity, respectively. T_{max} was the time of maximum observed concentration and C_{max} was the maximum observed concentration at T_{max}. C_{max} and T_{max} were obtained directly from the 5-ISMN plasma concentration-time curve. $t_{1/2}$ was estimated using the formula $t_{1/2} = 0.693/k_{10}$. The relative bioavailability (F) of the test tablets to the reference tablets was calculated using the following equation:

$$F = AUC_{0-\tau(\text{test})}/AUC_{0-\tau(\text{reference})} \times 100\%$$

The appropriate compartmental model was optimized by adopting Akaike's information criterion (Akaike 1981). Data fitting of cumulative release was performed to estimate the most probable release kinetics in SPSS 16.0 (SPSS Inc., Chicago, IL., U.S.A.).

Nonparametric test (Wilcoxon rank sum test) was used to evaluate the significance of T_{max}. Bilateral one-side test was performed on the log-transformed data of AUC and C_{max} to assess the bioequivalence of the two formulations. If the 90 % confidence interval for AUC and C_{max} were within the statistical interval (0.80 - 1.25) and (0.75 - 1.33) respectively, the two formulations would be considered bioequivalent.

References

Abshagen U, Betzien G, Endele R, Kaufmann B (1981) Pharmacokinetics of intravenous and oral isosorbide-5-mononitrate. Eur J Clin Pharmacol 20: 269–275.

Akaike H (1981) Likelihood of a model and information criteria. J Econom 16: 3–14.

Amidon G L, Lennernas H, Shah V P, Crison J R (1995) A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 12: 413–420.

Baidya S, Bedi S, Gupta BK (1999) Preparation and biopharmaceutical evaluation of nitrofurantoin loaded Eudragit RS100 and Eudragit RL 100 micropellets. Boll Chim Farm 138: 211–216.

Bonn R (1988) Sustained-release isosorbide mononitrate (50 mg): optimization of a once-daily dosage form for long-term treatment of angina pectoris. Am J Cardiol 61: 12E-14E.

Chevalier E, Viana M, Artaud A, Haddouchi S, Chulia D (2009) A novel application of the T-cell for flow-through dissolution: The case of bioceramics used as ibuprofen carrier. Talanta 77: 1545–1548.

Fotaki N, Symillides M, Reppas C (2005) *In vitro* versus canine data for predicting input profiles of isosorbide-5-mononitrate from oral extended release products on a confidence interval basis. Eur J Pharm Sci 24: 115–122.

Kramer W G (1994) Absorption of isosobide-5-mononitrate at specific sites in the gastrointestinal tract. J Clin Pharmacol 34: 1218–1221.

Li Z, He X, Gao X, Xu Y, Wang Y, Gu H, Ji R, Sun S (2011) Study on dissolution and absorption of four dosage forms of isosorbide mononitrate: Level A *in vitro–in vivo* correlation. Eur J Pharm Biopharm 79: 364–371.

Li X, Jiang Q, Du L, Wang C, Chi Q (2012) *In vitro* and *in vivo* evaluation of novel osmotic pump tablets of isosorbide-5-mononitrate containing polyvinyl pyrrolidone (PVP) for controlled release. Pharmazie 67: 695–700.

Lin SY (1993) Release kinetics of drug from different combined ratios of micropellets. J Microencapsul 10: 319–322.

Lopes C M, Lobo J M S, Pinto J F, Costa P (2006) Compressed mini-tablets as a biphasic delivery system. Int J Pharm 323: 93–100.

Marucci M, Ragnarsson G, Nilsson B, Axelsson A (2010) Osmotic pumping release from ethyl–hydroxypropyl–cellulose-coated pellets: A new mechanistic model. J Control Release 142: 53–60.

Ofori-Kwakye K, Fell J T (2001) Biphasic drug release: the permeability of films containing pectin, chitosan and HPMC. Int J Pharm 226: 139–145.

Parker J D, Parker J O (1998) Nitrate therapy for stable angina pectoris. New Eng J Med 338: 520–531.

Pastera J, Vysloužil L, Kvetina J (2004) Determination of isosorbide-5-mononitrate in human plasma by high-resolution gas chromatography. J Chromat B 800: 271–274.

Thomas GR, DiFabio JM, Gori T, Parker JD (2007) Once daily therapy with isosorbide-5-mononitrate causes endothelial dysfunction in humans. J Am College Cardiol 49: 1289–1295.

Verma R K, Kaushal A M, Garg S (2003) Development and evaluation of extended release formulations of isosorbide mononitrate based on osmotic technology. Int J Pharm 263: 9–24.

Walker J M, Curry P V L, Bailey A E, Steare S E (1996) A comparison of nifedipine once daily (Adalat LA), isosorbide mononitrate once daily, and isosorbide dinitrate twice daily in patients with chronic stable angina. Int J Cardiol 53: 117–126.

Wang Y B, Nedelman J (2002) Bias in the Wagner-Nelson estimate of the fraction of drug absorbed. Pharm Res 19: 470–476.

Wei X, Sun N, Wu B, Yin C, Wu W (2006) Sigmoidal release of indomethacin from pectin matrix tablets: effect of in situ crosslinking by calcium cations. Int J Pharm 318: 132–138.

Wu B, Chen Z, Wei X, Sun N, Lu Y, Wu W (2007) Biphasic release of indomethacin from HPMC/pectin/calcium matrix tablet: I. Characterization and mechanistic study. Eur J Pharm Biopharm 67: 707–714.