European Journal of Pharmaceutics and Biopharmaceutics 79 (2011) 364–371

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Study on dissolution and absorption of four dosage forms of isosorbide mononitrate: Level A *in vitro–in vivo* correlation

Zi-qiang Li [a], Xin He [a,b,*], Xiumei Gao [b,c], Yan-yan Xu [a], Yue-fei Wang [b,c], Hui Gu [a], Rui-feng Ji [a], Shu-jun Sun [a]

[a] Faculty of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, China
[b] Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin, China
[c] Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin, China

ARTICLE INFO

Article history:
Received 13 December 2010
Accepted in revised form 27 April 2011
Available online 5 May 2011

Keywords:
Isosorbide mononitrate (ISMN)
Drug dissolution/absorption simulating system (DDASS)
In vitro–in vivo correlation (IVIVC)
Bioavailability (BA)
Immediate-release (IR)
Sustained-release (SR)

ABSTRACT

The objective of the present study was to develop a novel *in vitro* system to simulate the process of dissolution and permeation of oral solid dosage forms *in vivo*, and to establish a correlation between *in vitro* permeation and *in vivo* absorption that could predict the bioavailability (BA) and bioequivalence (BE) of congeneric products. The *in vitro* dissolution and absorption kinetics of four dosage forms of isosorbide mononitrate (ISMN) were evaluated by the USP basket/paddle system and drug dissolution/absorption simulating system (DDASS). The corresponding pharmacokinetic study was performed in beagle dogs. A comparative study was carried out between the classical and the novel method to estimate the effectiveness of the modified DDASS in simulating the course of dissolution and absorption *in vivo*. Indeed, the correlation coefficients of *in vitro* dissolution and *in vivo* absorption obtained from DDASS and dogs were higher. Moreover, a higher level A *in vitro–in vivo* correlation (IVIVC) between DDASS permeation and dog absorption was established, with correlation coefficients of 0.9968, 0.9872, 0.9921, and 0.9728. The DDASS method was more accurate at modeling the process of dissolution and absorption *in vivo* for both immediate-release (IR) and sustained-release (SR) dosage forms of ISMN.

1. Introduction

A comprehensive guide for applying *in vitro–in vivo* correlation (IVIVC) models as a surrogate for *in vivo* bioequivalence (BE) testing during the initial drug approval process (or certain pre- or post-approval changes) was recently presented [1]. In addition, based on drug solubility and permeability, the Biopharmaceutics Classification System (BCS) was recommended [2]. The BCS provides a basis for predicting the likelihood of achieving a successful IVIVC. The term IVIVC previously appeared in the pharmaceutical literature [3–5] as a result of the awareness of the concepts of bioavailability (BA) and of *in vitro* dissolution rate determinations.

Many groups have developed methods for predicting intestinal absorption of drugs using artificial membranes, cultured cells, isolated tissues, and organ perfusion [6]. However, these transport studies used completely dissolved drug solutions, not oral solid formulations. Therefore, Ginski and co-workers [7] reported a continuous dissolution/Caco-2 system to predict the dissolution and absorption of solid formulations prior to human studies. Nonetheless, the method did not model the drastic pH changes in gastrointestinal (GI) tract. Hence, Kobayashi et al. [8] developed an *in vitro* system for the prediction of drug absorption that took into account the dissolution of solid drugs and pH changes in the GI tract. Moreover, He et al. [9–12] established six models with different gastric acidities and applied rat intestines to the *in vitro* system to evaluate the absorption of powders or grinded tablets. Meanwhile, Kataoka et al. [13,14] invented a dissolution/permeation system to which they applied an amount of drug corresponding to the clinical dose. Motz et al. [15,16] used a flow through cell to determine dissolution and permeation concomitantly. Those studies and devices have generated the useful information in considering the dissolution–permeation relationship in oral drug absorption. However, only a few of them allow the analysis of complete oral dosage forms rather than only non-formulated compounds. Consequently, we made some modifications [17] to the classical model system of Kobayashi et al. [8], with a basket installed in the drug-dissolving vessel and a two-stage filtering system.

The modified system is called drug dissolution/absorption simulating system (DDASS) and is presented schematically in Fig. 1. The work had been done in our previous research to investigate the necessity to modify the DDASS, and the results have been published [18]. In the research, salvianolic acid B sustained-release tablet was chosen as a model dosage form. A comparative study was carried out between the USP II method and DDASS method with and without modification. The results showed a better

* Corresponding author. Faculty of Chinese Materia Medica, Tianjin University of Traditional Chinese Medicine, Tianjin, China. Tel.: +86 22 5959 6232; fax: +86 22 5959 6153.
E-mail address: hexintn@yahoo.com (X. He).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.04.015

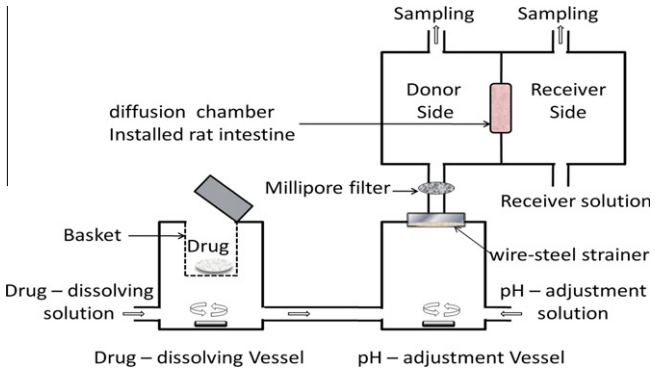

Fig. 1. Scheme of the modified drug dissolution/absorption simulating system (DDASS). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

correlation between USP II method and DDASS method with modification. Therefore, in the present research, four commercial dosage forms of isosorbide mononitrate (ISMN) (Xinkang® tablets, Shansu® capsules, Imdur® sustained-release tablets, and Elantan® sustained-release capsules) were chosen. Isosorbide mononitrate is an organic nitrate used in the prophylaxis of angina pectoris [19]. As DDASS is expected to imitate the process of dissolution and permeation of solid formulation simultaneously [8], we expect to make a provisional biopharmaceutical classification of ISMN. A comparative study was carried out between the classical and the novel method to estimate the efficacy of the modified DDASS in simulating the continuous dynamic characteristics and the stress state of dosage forms in the process of dissolution and absorption *in vivo*. In addition, the BE of each ISMN dosage form was evaluated and compared between DDASS and beagle dogs. It is expected that we can establish a better level A IVIVC between DDASS permeation and dog absorption than between USP basket/paddle dissolution and dog absorption for each formulation.

2. Materials and methods

2.1. Chemicals and drug dosage forms

Isosorbide mononitrate and Isosorbide-2-nitrate were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Isosorbide Mononitrate Tablets (ISMN-T, 20 mg, Xinkang®), Isosorbide Mononitrate Capsules (ISMN-C, 20 mg, Shansu®), Isosorbide Mononitrate Sustained-Release Tablets (ISMN-SRT, 60 mg, Imdur®), and Isosorbide Mononitrate Sustained-Release Capsules (ISMN-SRC, 50 mg, Elantan®) were provided by Lunan Pharma Group (Shandong, China), Yangtze River Pharma Group (Jiangsu, China), Astrazeneca Co., Ltd. (UK), and Schwarz Pharma Co., Ltd. (Germany), respectively. HPLC-grade acetonitrile was provided by Concord Co., Ltd. (Tianjin, China). Ultra-pure water was prepared using a Milli-Q Synthesis system (Millipore, Billerica, MA); MES hydrate, HEPES, and D-(+)-glucose were purchased from Sigma Co., Ltd. (USA). All other chemicals used in the study were of analytical grade.

2.2. Animals

Male Wistar rats, weighing 280–320 g, were provided by Chinese Academy of Medical Sciences Institute of Radiation Medicine (Tianjin, China). Rats were fasted for 12 h before experiments but had free access to water. During experiments, rats were anesthetized with ether and the jejunum of each rat (2.0–2.5 cm) was removed.

Male purebred beagle dogs, weighing 8.5 ± 0.5 kg, were obtained from the Chinese PLA Academy of Military Medical Science Laboratory Animal Center (Beijing, China). Throughout the experiments, dogs were housed one per cage for at least 10 days before experiments and the kennel temperature was maintained at 25 ± 2 °C. Expanded feed for dogs was purchased from KeAoXieLi Food Co., Ltd. (Beijing, China). The Academy of Military Medical Science Institutional Animal Care and Use Committee (Certificate No. SCXK-2007-004) approved this animal study.

2.3. Drug dissolution using apparatus basket/paddle

The drug dissolution characteristics were investigated with a dissolution apparatus (SOTAX AT7, Switzerland) according to the procedure described in USP [20]. Dissolution testing apparatus for different ISMN dosage forms are listed in Table 1. The dissolution medium (900 mL) was deaerated and maintained at 37.0 ± 0.5 °C. At different time intervals, 2 mL samples were withdrawn and filtered through 0.45 μm Millipore filters. After each sampling, an equivalent volume of fresh medium was replaced. Each dissolution profile was obtained from six replications.

2.4. Drug dissolution and permeation using DDASS

As shown in Fig. 1, our system for predicting drug absorption modeled drug dissolution and pH changes in GI tract [17], with some modification. The modified DDASS has broader application for tablets and capsules. First, we installed a basket in the drug-dissolving vessel (DDV; modeled stomach) to carry the complete oral dosage forms. The vertical center line of the basket passes through the axis of the vessel so that drug dosage forms will be in the middle of the DDV. On the one hand, the eccentricity ratio of the magnetic stir bar will not be changed since it does not collide with a dosage form, so the hydrodynamic effects of dissolution medium will not vary. On the other hand, the complete oral dosage forms will stay at the same position and maintain the same solid–liquid interface dynamic effects. Secondly, we added a wire-steel strainer on the top of the pH-adjustment vessel (PAV; model stomach) as a first filtering system to prevent escape of undissolved particles from the PAV. A Millipore filter was installed between the PAV and side-by-side diffusion champer as a second filtering system to further purify the drug solution. The compositions of the drug-dissolving solution, pH-adjustment solution, and receiver solution are shown in Table 2. The flow rate of each solution (0.5 mL/min) was controlled using a peristaltic pump. Though the volume of each vessel was fixed (10 mL), the flow rate of the solution had been justified as reported in our previous study [9].

For ISMN-SR dosage forms, we first collected eluted solutions of the donor compartment every 10 min over a period of 12 h. In the study of the permeation properties of four commercial ISMN dosage forms, a jejunum removed from a rat was mounted between the donor and the receiver compartments. Under the condition of bubbling with O_2–CO_2 (95:5) mixture gas, solutions eluted into the donor compartment and to the receiver compartment were collected every 10 min over a period of 5 h. Each dissolution profile was obtained from three trials.

Table 1
Dissolution conditions of four different dosage forms of ISMN.

Drugs	Apparatus	Rotation speed (r/min)	Time intervals (h)
ISMN-T	Paddle	50	0.08, 0.17, 0.25, 0.33, 0.42, 0.5
ISMN-C	Basket	100	0.08, 0.17, 0.25, 0.33, 0.42, 0.5
ISMN-SRT	Paddle	50	1, 2, 3, 4, 5, 6, 8, 10, 12
ISMN-SRC	Basket	100	1, 2, 3, 4, 5, 6, 8, 10, 12

Table 2
Composition of flowing solutions (mM) [9].

Component	Drug-dissolving solution	pH-adjustment solution[a]	Acceptor solution[b]
KCl	–	10.7	5.37
KH_2PO_4	–	0.88	0.44
NaCl	61.4	199.2	137.0
Na_2HPO_4	–	0.68	0.34
D-Glucose	50.0	–	25.0
$CaCl_2$	2.52	–	1.26
$MgSO_4$	0.81	–	0.41
MES	–	40.0	–
HEPES	–	–	10.0
HCl	13.6	–	–
NaOH	–	13.6	–

[a] pH adjusted to 6.0 with Tris before addition of NaOH.
[b] pH adjusted to 7.4 with Tris.

2.5. HPLC analysis of dissolution and permeation in vitro

The concentrations of ISMN samples *in vitro* were determined with a validated HPLC method. A TC–C_{18} column (5 μm, 4.6 × 150 mm, Agilent, USA) with an analytical TC–C_{18} guard column (5 μm, 4.6 × 12.5 mm, Agilent, USA) was maintained at 30 °C. The analytical mobile phase consisted of water and acetonitrile in an 80:20 (v/v) ratio. The flow rate was 0.8 mL/min. The injection volume was 10 μL, and ISMN was detected by absorbance at 220 nm.

2.6. Kinetic analysis of dissolution characteristics

The dissolution test is one of the techniques most widely used in the characterization of drugs and in quality control of drug dosage forms. In order to explore the drug release mechanism of the commercial SR dosage forms, the dissolution data were processed by zero-order, first-order, Higuchi, Hixson-Crowell, and Ritger–Peppas models [21,22]. The value of *r* stands for the goodness-of-fit of the corresponding model. The higher of the goodness, the closer the model fit the process of dissolution for solid dosage forms. The release mechanism of ISMN-SR was evaluated by the optimal mathematics model. The concentration of ISMN can also be predicted by the mathematics model at any time. The correlation between DDASS elution data and the classical dissolution experiment was studied. A point-to-point relationship will be established to evaluate the release characteristics of ISMN-SR dosage forms in DDASS method and classical dissolution methods.

2.7. Pharmacokinetic studies in beagle dogs

The four commercial ISMN dosage forms were divided into the two groups IR and SR. We evaluated each group in six dogs according to a single-dose randomized cross-over design. There was at least a 7-day washout period between each dosing. The dogs were fasted for at least 12 h prior to the experiment, with free access to water and food 4 h after drug administration. Blood samples (2 mL) were collected from the cephalic vein of the front leg at 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 h after IR doses and at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 12, 18, 24, 36, 48 h after SR doses. These samples were immediately centrifuged at 3500 rpm for 15 min and stored at −20 °C until analysis.

To an aliquot of plasma sample (500 μL) in a glass centrifuge tube, 100 μL of phosphate-buffered saline solution (pH 7.8) and 10 μL of 10 μg/mL isosorbide-2-nitrate (the internal standard) were added. After vortex mixing for 30 s, 4 mL of ethyl acetate was added and vortexed for 5 min. The organic layer was separated by centrifugation at 3500 rpm for 10 min and evaporated to dryness under a gentle stream of nitrogen at 40 °C. The residue was dissolved with 100 μL of ethyl acetate and then centrifuged.

2.8. GC analysis of in vivo pharmacokinetics

An Agilent J&W Scientific 6890 N Network Gas Chromatograph (GC) system (Agilent Technologies, USA) equipped with a 63 Ni-Electron-Capture Detector (ECD) and an Agilent 7683 Series Injector (Agilent Technologies, USA) was used, together with a 30 m × 0.32 mm HP-5 Gas Chromatography Column with a film thickness of 0.25 μm (Agilent Technologies, USA). High purity nitrogen at a flow rate of 2 mL/min was used as the carrier gas. The temperature of the inlet was set at 180 °C. The temperature of ECD was set at 180 °C with a gas flow of 40 mL/min. The column temperature was maintained for 8 min at 160 °C. A GC ChemStation (Agilent Technologies, USA) was used for controlling the GC apparatus, and data acquisition and analysis.

2.9. In vitro–in vivo correlation

The term IVIVC refers to the establishment of a predictable relationship between some biological process (or a parameter derived from a biological property produced by a dosage form) and a physicochemical property or characteristic of the same dosage form. The correlation between *in vitro* dissolution and *in vivo* absorption was studied to evaluate the release characteristics of ISMN dosage forms obtained from the DDASS and basket/paddle methods, and to determine which more accurately modeled *in vivo* BA. A level A IVIVC represents a point-to-point relationship between *in vitro* dissolution and *in vivo* input rate of the drug, as prescribed in the pharmacopoeia [23,24].

The percentage fraction absorbed (F_a) of the different dosage forms was calculated by the Wagner–Nelson Eq. (1) or by the Loo–Riegelman Eq. (2) for both a single compartment model and the two-compartment model [25].

$$F_a = (C_t + k_{10} * AUC_{0-t})/(k_{10} * AUC_{0-\inf}) \times 100\% \quad (1)$$

$$F_a = (C_t + k_{10} * AUC_{0-t} + X_t/V_c)/(k_{10} * AUC_{0-\inf}) \times 100\% \quad (2)$$

In both equations, C_t is the concentration at time point t, k_{10} is the elimination rate of the dosage form, AUC_{0-t} is the area under the curve from zero to time t, and $AUC_{0-\inf}$ is the area under the curve from zero to infinity. In Eq. (2), X_t is the dosage of peripheral compartment at the time t. The percentage of dissolution and F_a at corresponding time points were fitted into a regression equation by least square method. The regression coefficient was the index of correlation between *in vitro* release and *in vivo* absorption.

The predicted concentrations obtained from the DDASS method were compared with the *in vivo* observed concentrations of different ISMN dosage forms. Parameter AUC at different time intervals was calculated over a period of 5 h. We used the linear trapezoidal rule to summate each pair of consecutive points in the data set. If a selected partial area had an endpoint that was not in the data set, then the Linear Trapezoidal Linear/Log Interpolation rule was used to add a concentration value for that endpoint. The partial AUC_{0-t} values of *in vitro* prediction and *in vivo* observation at time intervals were fitted to a regression equation by the least square method.

2.10. Data analysis and statistics

The dissolution and absorption kinetic parameters of ISMN dosage forms in DDASS and the pharmacokinetic parameters in beagle dogs were determined using Phoenix WinNonlin version 6.1 (Pharsight Co., Ltd., USA), assuming a noncompartmental analysis model. Here, λ_z was first-order rate constant associated with the terminal (log-linear) portion of the curve, T_{max} was time of maximum observed concentration, and C_{max} was the maximum observed concentration at T_{max}. The value of AUC_{last} was the area under the curve while MRT_{last} was mean residence time from the

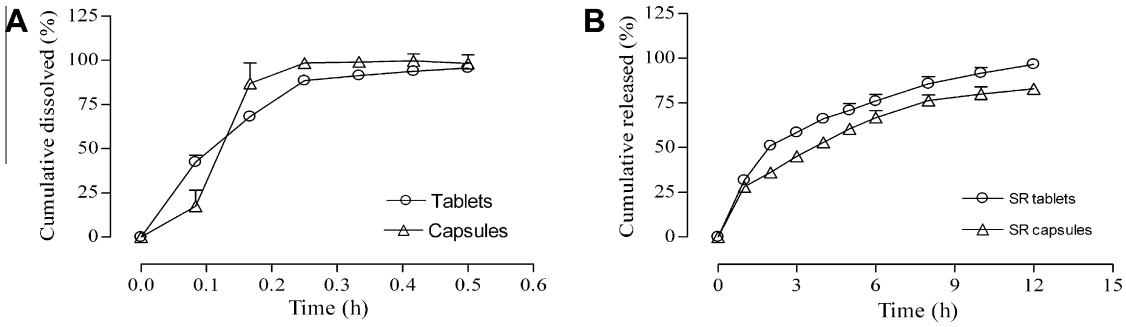

Fig. 2. Dissolution profiles of ISMN-IR formulations (A) and ISMN-SR formulations (B) in basket/paddle methods. Each point represents the mean ± SD of six experiments.

time of dosing to the time of the last measurable concentration. The appropriate compartmental model was optimized by adopting Akaike's information criterion [26]. Data fitting of cumulative release was performed to estimate the most probable release kinetics in SPSS 11.5. Independent measured *t*-test was used to analyze the differences between the mean values. The *p*-value was considered statistically significant when less than 0.05.

3. Results

3.1. Dissolution results using apparatus basket/paddle methods

The dissolution profiles of ISMN dosage forms obtained from USP apparatus paddle/basket methods are shown in Fig. 2. Isosorbide mononitrate dissolved almost instantaneously with dissolution of more than 98% for ISMN-T and 91% for ISMN-C within 20 min (Fig. 2A). Release testing of ISMN-SRT and SRC yielded a release of 29.09%, 28.05% at 1 h and 96.59%, 82.82% at 12 h (Fig. 2B). Isosorbide mononitrate SR dosage forms demonstrated slower release compared with IR dosage forms.

3.2. Dissolution and permeation results using DDASS methods

The time courses of ISMN-IR and ISMN-SR elution into the donor compartment are shown in Fig. 3A and B, and their corresponding cumulative elution profiles are given in Fig. 3C and D. Cumulative elutions for ISMN-T and ISMN-C were 4.89% and 5.01% at 20 min and plateaued at 91.29% and 95.83% in DDASS after 2 h. In contrast, cumulative elutions reached similar levels of 91.43% and 98.99% in paddle/basket methods at 20 min. For ISMN-SRT and ISMN-SRC, the DDASS method yielded a cumulative elution of 31.35% and 29.65% at 2 h, but reached lower endpoints of 86.63% and 50.39% at 12 h, while the conventional methods yielded a cumulative elution of only 51.12% and 36.03% at 2 h. The elution course of each ISMN dosage forms observed in DDASS was characterized by a slower rise compared with conventional methods.

Fig. 4 illustrates the time courses of cumulative permeation to the receiver compartment for ISMN-IR and ISMN-SR dosage forms in DDASS. Table 3 displays kinetic parameters of ISMN eluted into the donor compartment and permeation to the receiver compartment. Compared with ISMN-T, the average relative bioavailability was 101.23% for ISMN-C, 56.52% for ISMN-SRT, and 40.07% for ISMN-SRC in the donor side, and 114.79%, 57.45%, and 51.06% in the receiver side within 5 h. For ISMN-SRT and ISMN-SRC, cumulative elutions of 58.51% and 38.48% were observed in the donor compartment at 5 h, whereas ISMN-IR dosage forms dissolved completely within 4 h. With regard to T_{max} and C_{max}, ISMN-SR dosage forms did not show significant differences from ISMN-IR dosage forms ($p > 0.05$). The reasons were probably that the inter-vessel transport of DDASS was rate-limiting and ISMN-SR contained 30% IR.

3.3. Kinetic analysis of dissolution characteristics

The ISMN-SR dosage forms contained 30% IR, so we estimated the elution kinetic characteristics of ISMN-SR dosage forms from DDASS after 2 h. At 2 h, the cumulative elution of ISMN-SRT and ISMN-SRC were 31.25% and 29.66% (Fig. 3D). The correlation between data from DDASS and those from classical dissolution experiment have been compared. As for ISMN-SR dosage forms, the equation and coefficient of linear regression is $F_{d(\mathrm{DDASS})} = 1.12F_{d(\mathrm{paddle})} - 21.57$ ($r = 0.9969$) for ISMN-SRT, and $F_{d(\mathrm{DDASS})} = 0.53F_{d(\mathrm{basket})} + 6.53$ ($r = 0.9469$) for ISMN-SRC. Besides, based on the value of n obtained by the Ritger–Peppas equation, the release mechanisms for both ISMN-SR dosage forms were consistent with Fickian diffusion ($n < 0.5$). Table 4 shows that the optimal elution equation for each ISMN-SR dosage form best fits a first-order kinetic model in the DDASS method, just like the basket/paddle method.

3.4. Pharmacokinetic characteristics in beagle dogs

Fig. 5 shows the mean plasma concentrations of ISMN after a single oral administration of the ISMN-IR dosage forms (Fig. 5A) and the ISMN-SR dosage forms (Fig. 5B) to beagle dogs. The pharmacokinetic parameters are summarized in Table 5. As shown in Fig. 5A and Table 4, the drug plasma concentrations after oral administration of ISMN-IR dosage forms increased rapidly and reached C_{max} at 1 h. The results were consistent with the receiver compartment permeation obtained from DDASS (Table 3), with a T_{max} value of 1.33 ± 0.19 h and 1.17 ± 0.24 h for ISMN-T and ISMN-C, respectively. No significant differences ($p > 0.05$) were observed for the C_{max} values obtained for the four different ISMN dosage forms, whether in dogs or in DDASS due to the 30% immediate release of the ISMN-SR dosage forms.

Based on the *two one-sided t-test* procedure, the calculated confidence interval should fall within a BE limit, usually 80–125% for the ratio of the test dosage forms. Compared with ISMN-T, the relative BA values of ISMN-C, SRT, SRC were 108.33 ± 8.61%, 94.33 ± 4.12%, and 109.21 ± 16.11%, respectively, and they all met the requirement of the guidance [27] mentioned above. Parameters MRT_{last} and λ_z of obtained for ISMN-SR dosage forms showed significant differences ($p < 0.05$) in terms of their 70% SR contents compared with ISMN-IR dosage forms.

3.5. In vitro–in vivo correlation

The optimization one compartment model was determined for ISMN dosage forms after validation by Akaike's information criterion. The terminal elimination rate constants for ISMN-T, ISMN-C, SRT, and SRC were 0.89 ± 0.03, 0.61 ± 0.04, 0.15 ± 0.01, and

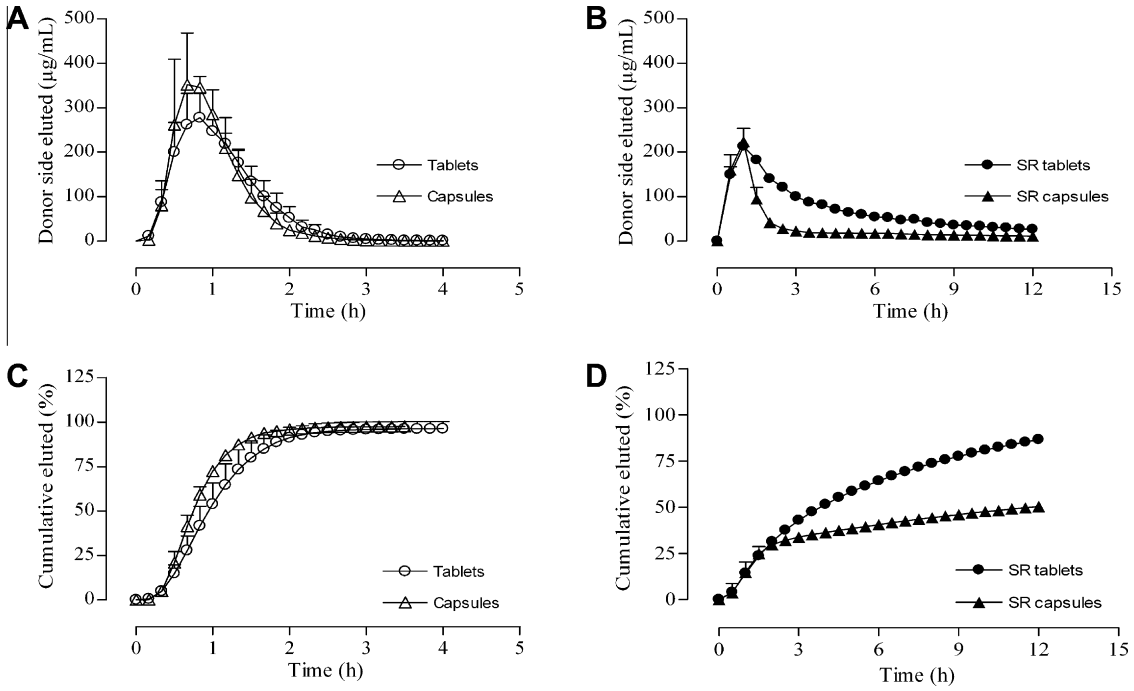

Fig. 3. Time courses of elution into the donor compartment from ISMN-IR formulations (A) and ISMN-SR formulations (B) in DDASS, and corresponding cumulative elution profiles (C) and (D). Each point represents the mean ± SD of three experiments.

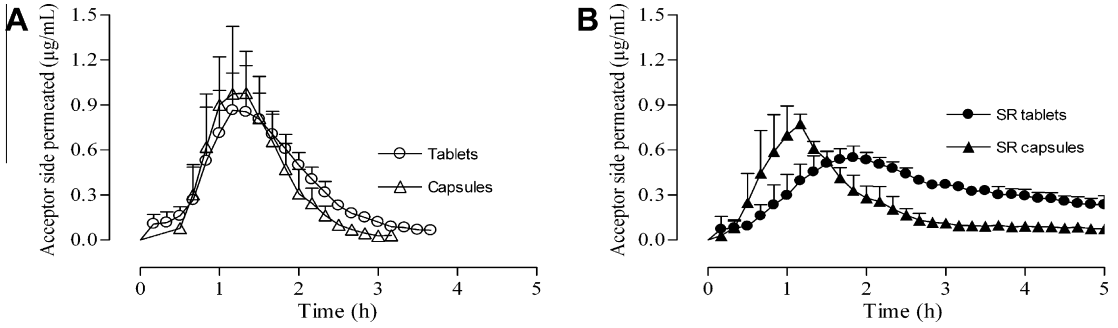

Fig. 4. Time courses of permeation to the receiver compartment of ISMN-IR formulations (A) and ISMN-SR formulations (B) in DDASS methods. Each point represents the mean ± SD of three experiments.

0.25 ± 0.02 (Table 5). With the calculated F_a, the regression equation and coefficient of correlation between basket/paddle dissolution and dog absorption were summarized (Table 6), as well as between DDASS elution and dog absorption. More than 90% was dissolved within 20 min *in vitro*, so no correlation could be established for IR dosage forms due to their rapid dissolution in the basket/paddle apparatus. However, a level A IVIVC was established with correlation coefficients of 0.9903 and 0.9785 for ISMN-T and ISMN-C, respectively, between DDASS and dogs. Furthermore, a better goodness-of-fit could be obtained for ISMN-SRT and SRC between DDASS and dogs than between basket/paddle and dogs (Table 6).

The regression of AUC data obtained from the plot of ISMN plasma concentration vs. time (Fig. 5) and that of permeated ISMN concentration vs. time (Fig. 4) for four different dosage forms at predetermined time points are shown in Table 7. The correlation coefficient (r) is a criterion to evaluate the fit of the linear regression equation with the data. The closer is r to 1, the better is the correlation between DDASS permeation and dog absorption. A better level A IVIVC could be obtained with a higher correlation coefficient, $r > r_{5,0.001}$ ($r_{5,0.001} = 0.951$), compared with the correlation between *in vitro* dissolution and *in vivo* absorption (Table 6) for each of the four ISMN dosage forms.

4. Discussion

4.1. Provisional BCS classification of ISMN

The BCS is a scientific framework [2] for classifying drug substances based on their aqueous solubility and intestinal permeability. In this study, the maximal concentration of ISMN was 395.49 ± 29.05 μg/mL (Table 2) after undergoing drastic pH change from 1.8 to 6.8 in DDASS. A 60 mg ISMN dose could be dissolved in a dissolution media volume of 151.71 ± 11.14 mL. The FDA drug evaluation and research center has reported that ISMN is freely soluble in water. Therefore, these results suggest that ISMN is a drug that has "high solubility." The cumulative intestinal permeation values for ISMN-T, ISMN-C, SRT, and SRC were 0.15 ± 0.03%, 0.17 ± 0.02%, 0.08 ± 0.003%, and 0.07 ± 0.003% in DDASS, respectively. In addition, He et al. [9] reported that a cumulative

Table 3
Kinetic parameters of ISMN elution into the donor compartment and permeation to the receiver compartment for the different ISMN dosage forms.

Compartment	Parameter	ISMN-T	ISMN-C	ISMN-SRT	ISMN-SRC
Donor side	T_{max} (h)	0.79 ± 0.08	0.75 ± 0.17	0.88 ± 0.08	0.67 ± 0.17
	C_{max} (μg/mL)	280.60 ± 59.91	395.49 ± 29.05*	216.04 ± 18.41	291.81 ± 37.53
	AUC_{last}/D (μg h/mL/mg)	16.17 ± 0.73	16.37 ± 0.10	9.14 ± 0.27*	6.48 ± 0.15*
Receiver side	T_{max} (h)	1.33 ± 0.19	1.17 ± 0.24	1.88 ± 0.25	1.11 ± 0.09
	C_{max} (μg/mL)	0.93 ± 0.50	1.04 ± 0.09	0.57 ± 0.04	0.82 ± 0.07
	AUC_{last}/D (μg h/mL/mg)	0.047 ± 0.025	0.054 ± 0.005	0.027 ± 0.001*	0.024 ± 0.001*

Each value is the mean ± SD of three experiments.
* $p < 0.05$: significantly different from ISMN-T.

Table 4
Release kinetic parameters and correlation coefficients of each equation for ISMN-SR formulations.

Release apparatus	Release models	ISMN-SRT		ISMN-SRC	
		Equation	R	Equation	R
Basket/ Paddle	Zero-order	$F_t = 0.0541t + 0.3889$	0.9474	$F_t = 0.0513t + 0.2975$	0.9533
	First-order	$\mathrm{Ln}(1 - F_t) = -0.2539t - 0.0595$	0.9881	$\mathrm{Ln}(1 - F_t) = -0.1374t - 0.215$	0.9953
	Higuchi	$F_t = 0.2569t^{0.5} + 0.1165$	0.9881	$F_t = 0.2404t^{0.5} + 0.0458$	0.9879
	Ritger-Peppas	$\mathrm{Ln}\ F_t = 0.4474\mathrm{Ln}\ t - 1.0834$	0.9818	$\mathrm{Ln}\ F_t = 0.47\mathrm{Ln}\ t - 1.2914$	0.9934
	Hixson-Crowell	$(1 - F_t)^{1/3} = -0.0478t + 0.9031$	0.9957	$(1 - F_t)^{1/3} = -0.0325t + 0.9111$	0.9816
DDASS	Zero-order	$F_t = 0.0514t + 0.3077$	0.9788	$F_t = 0.0193t + 0.2834$	0.9927
	First-order	$\mathrm{Ln}(1 - F_t) = -0.1586t - 0.0788$	0.9998	$\mathrm{Ln}(1 - F_t) = -0.0331t - 0.3156$	0.9991
	Higuchi	$F_t = 0.2622t^{0.5} - 0.0152$	0.9952	$F_t = 0.0977t^{0.5} + 0.1667$	0.9987
	Ritger-Peppas	$\mathrm{Ln}\ F_t = 0.4924\mathrm{Ln}\ t - 1.3405$	0.9965	$\mathrm{Ln}\ F_t = 0.2889\mathrm{Ln}\ t - 1.4116$	0.998
	Hixson-Crowell	$(1 - F_t)^{1/3} = -0.0358t + 0.9314$	0.9977	$(1 - F_t)^{1/3} = -0.0092t + 0.8982$	0.9958

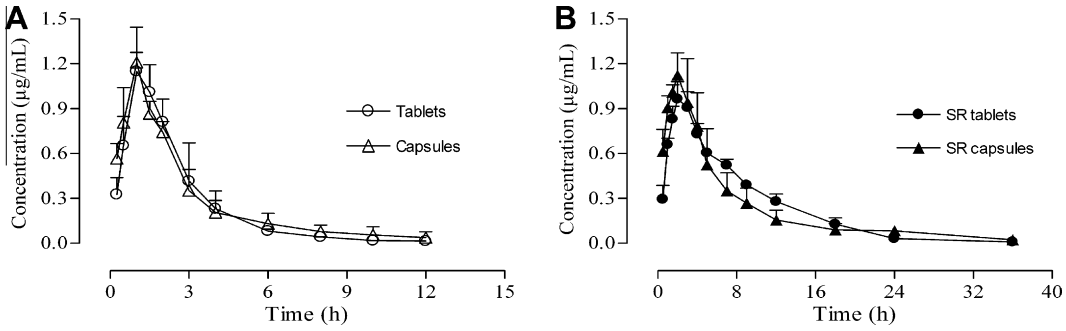

Fig. 5. Profiles of plasma concentration after oral administration of ISMN-IR formulations (A) and ISMN-SR formulations (B) to beagle dogs. Each point represents the mean ± SD of six experiments.

Table 5
Pharmacokinetic parameters of ISMN after a single oral administration of the four tested ISMN dosage forms.

Parameters	ISMN-T	ISMN-C	ISMN-SRT	ISMN-SRC
T_{max} (h)	1.00 ± 0.00	1.00 ± 0.00	2.67 ± 0.58	2.33 ± 0.58
C_{max} (μg/mL)	1.15 ± 0.13	1.21 ± 0.24	0.98 ± 0.11	1.13 ± 0.16
AUC_{last} (μg/mL h)	3.00 ± 0.34	3.25 ± 0.28	8.49 ± 0.35	8.19 ± 2.22
AUC_{inf} (μg/mL h)	3.04 ± 0.33	3.76 ± 0.87	8.56 ± 0.36	8.49 ± 2.18
MRT_{last} (h)	2.49 ± 0.21	2.85 ± 0.37	7.74 ± 0.16*	7.46 ± 1.23*
λ_z (1/h)	0.79 ± 0.16	0.65 ± 0.18	0.14 ± 0.01*	0.25 ± 0.10*

Each value is the mean ± SD of six experiments.
* $p < 0.05$: significantly different from ISMN-T.

permeation across a rat intestine of more than 0.04% signified the drug was almost completely absorbed in humans. Therefore, ISMN has "high permeability." In general, the transport studies were performed under sink conditions and the P_{app} coefficients were calculated from the equation

$$P_{app} = (\Delta Q/\Delta t)/(A \cdot C) \quad (3)$$

where $\Delta Q/\Delta t$ is the steady-state flux (mol/s), A is the effective surface area of intestine (cm^2), and C is the initial concentration in the donor compartment at each time interval (mol/mL). The average P_{app} of ISMN, obtained from DDASS, was $(28.36 \pm 12.04) \times 10^{-6}$ cm/s. Drugs are completely absorbed in humans if their P_{app} values are more than 1×10^{-6} cm/s [28–30]. Nevertheless, other studies [13] proposed that drugs be completely absorbed if P_{app} were more than 5×10^{-6} cm/s. Furthermore, ISMN was essentially completely absorbed after oral administration with no first-pass metabolism [31,32]. In any case, ISMN can be regarded as a high-permeability drug. Considering that dinitrate, a generic drug product, is in the BCS I category [33,34], a provisional biopharmaceutical classification of ISMN could be predicted to be BCS I by using DDASS alone.

4.2. In vitro dissolution–in vivo absorption correlation

During the development of an innovative dosage form, it is necessary to establish an *in vitro* test method that can predict the course of drug release and absorption of products *in vivo*. In the present study, the release characteristics of different ISMN dosage forms and their corresponding "*in vitro* dissolution-dog absorption correlation" were investigated by using conventional methods and DDASS. For ISMN-IR dosage forms, there was a significant

Table 6
Regression equation and correlation coefficients between basket/paddle dissolution and dog absorption as well as between DDASS elution and dog absorption.

Drugs	Basket/paddle vs. dogs		DDASS vs. dogs	
	Equation	(R)	Equation	(R)
ISMN-T	–		$F_a = 0.74\ F_d + 16.45$	(0.9903)
ISMN-C	–		$F_a = 0.49\ F_d + 29.09$	(0.9785)
ISMN-SRT	$F_a = 0.47F_d + 52.70$	(0.8031)	$F_a = 0.72\ F_d + 44.99$	(0.8231)
ISMN-SRC	$F_a = 0.61\ F_d + 35.71$	(0.7828)	$F_a = 1.07\ F_d + 33.19$	(0.9549)

—: No correlation was established.

Table 7
The correlation between DDASS permeation and dog absorption for ISMN dosage forms.

Dosage forms	Equation	r
Tablets	$F_a = 1.58\ F_p + 0.11$	0.9968
Capsules	$F_a = 1.66\ F_p + 0.28$	0.9872
Sustained-release tablets	$F_a = 2.10\ F_p + 0.14$	0.9921
Sustained-release capsules	$F_a = 3.41\ F_p - 0.17$	0.9728

correlation between DDASS elution and dog absorption, whereas there was no dramatic correlation between basket/paddle dissolution and dog absorption (Table 6). Biowaivers of BA/BE studies for IR solid oral dosage forms *in vivo* were provided by the guidance [35]. To some extent, the level A correlation between DDASS elution and dog absorption of ISMN-IR dosage forms could be used to support the biowaiver policy for oral generic drugs. In recent years, IVIVC studies of IR dosage forms have been performed using different methods [36–38]. For ISMN-SR dosage forms, release kinetic characteristics were fit by first-order kinetics equations. In this study, both ISMN-SR dosage forms presented consistent release kinetics by conventional and DDASS methods (Table 4). On the other hand, the drug release mechanism was consistent with Fickian diffusion ($n < 0.5$). Some drug granules undergo a drastic pH change from DDV to PAV in DDASS, so the release exponent n for ISMN-SRC was only 0.2889, lower than obtained from a single medium in the basket method. Furthermore, a better IVIVC could be obtained for ISMN-SR dosage forms between DDASS dissolution and dog absorption than between paddle/basket dissolution and dog absorption (Table 6). The DDASS provided a condition that was much closer to the actual situation in the GI tract due to the added basket and the changed pH condition.

4.3. In vitro permeation–in vivo absorption correlation

Methods that more closely model the *in vivo* situation are less suitable for mass screening because they are more labor-intensive and consume more materials. The basket/paddle method is approved for investigation into drug release and solubility, but drug absorbability cannot be estimated. In contrast, DDASS provided not only *in vitro* dissolution information in the donor compartment but also permeation in the receiver compartment. Normally, effective drug constituents can be absorbed only through dissolution of the oral solid dosage form and permeation through GI tract. High solubility and dissolution does not always lead to high mean BA and BE, however. Amidon et al. [2] demonstrated that the fundamental events controlling oral drug absorption were the permeability of the drug through the GI membrane and the solubility/dissolution. Therefore, the correlation between *in vitro* permeation and *in vivo* absorption can be theoretically better than *in vitro* dissolution and *in vivo* absorption. Previous studies have systematically analyzed the dissolution and permeability of IR dosage forms [15,38] and the parameter level correlation between DDASS permeation and oral absorption [9,10]. In the present study, permeation and absorption profiles of each ISMN dosage form (Figs. 4 and 5) were observed in DDASS and in dogs. Moreover, a better level A correlation was obtained for each ISMN dosage form between DDASS permeation and dog absorption (Table 7) than between *in vitro* dissolution and dog absorption (Table 6). The established IVIVC was level A, thus confirming the efficacy of this *in vitro* model in simulating *in vivo* conditions. This kind of correlation is quite important since it represents a point-to-point relationship between *in vitro* permeation and *in vivo* input rate of drugs with different dosage forms.

4.4. Application outlook of the modified DDASS

Predictive scientific principles and methods to assess *in vivo* performances of pharmaceutical dosage forms based on *in vitro* studies are important to minimize costly animal and human experiments during drug development. An advantage of this DDASS is that the dissolution medium can be easily changed to any appropriate solution to assess different oral solid dosage forms. As far as poorly water-soluble drugs are concerned, a certain surfactant or cholalic acid could be added to the dissolution medium in order to increase dissolution rates [23,24]. Bile acids are secreted into the duodenum from the gallbladder, and the average concentration in human intestine was reported to be 5–15 mM [39,40]. Kataoka et al. [13] added a 5 mM concentration of sodium taurocholate to the apical solution for griseofulvin dissolution and permeation, which corresponded to the concentration during fasting conditions in human. In addition, dissolution media simulating the fasted and fed states *in vivo* could also be modeled to evaluate food impact on drug release. For instance, by modeling intestinal fluids in the fasted and fed states, as proposed by Dressman et al. [41,42], food effect on total absorption, not just on dissolution, could be monitored. Souliman et al. [43] simulated the impact of food intake on drug release and absorption using different conditions and established a level A IVIVC in fasted and fed states for the IR dosage forms. Moreover, a Caco-2/3A4 cells model [44,45], in which Caco-2 cells were stably transfected with the human CYP3A4 gene, could be established in our DDASS to evaluate the absorption and CYP3A4-mediated intestinal elimination of oral solid dosage forms. These studies are now under investigation and are the subject of future reports.

5. Conclusions

A better IVIVC was established between DDASS permeation and dog absorption for each ISMN dosage form than between *in vitro* dissolution and dog absorption. Furthermore, a provisional biopharmaceutical classification of ISMN as BCS I was predicted using the DDASS method alone. Therefore, DDASS demonstrated a high level of effectiveness in mimicking the process of dissolution and absorption *in vivo* for both the IR and SR dosage forms of ISMN. The DDASS could be used to forecast the *in vivo* bioavailability and bioequivalence of congeneric products. This novel method provides conditions that more accurately model the conditions in GI tract by adding a basket and a second filtering system. The DDASS also represents a new method to design more effective dosage forms and to optimize prescriptions.

Acknowledgments

This project was supported by Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT); The Foundation of The Ministry of Science and Technology of the People's Republic of China, No. 2009ZX09304-002 and 2009ZX09311-002;

National Administration of Chinese Traditional Medicine of China, No.200807051 and No. 200800630005.

References

[1] Food and Drug Administration: Guidance for Industry: Nonsterile Semisolid Dosage Forms, Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; *In vitro* Release Testing and *In vivo* Bioequivalence Documentation, US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research, 1997.

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

[3] J.E. Polli, G.S. Re, L.L. Augsburger, V.P. Shah, Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets, J. Pharm. Sci. 86 (1995) 690–700.

[4] J.E. Polli, J.R. Amidon, G.L. Amidon, Novel approaches to the analysis of *in vitro–in vivo* relationships, J. Pharm. Sci. 85 (1996) 753–760.

[5] G.S. Rekhi, N.D. Eddington, M.J. Fossler, P. Schwartz, L.J. Lesko, L.L. Augsburger, Evaluation of *in vitro* release rate and *in vivo* absorption characteristics of four metoprolol tartrate immediate-release tablet formulations, Pharm. Dev. Technol. 2 (1997) 11–24.

[6] A. Pagliara, M. Geinoz, S. Geinoz, P.A. Carrupt, B. Testa, Evaluation and prediction of drug permeation, J. Pharm. Pharmacol. 51 (1999) 1339–1357.

[7] M.J. Ginski, J.E. Polli, Prediction of dissolution–absorption relationships from a dissolution/Caco-2system, Int. J. Pharm. 177 (1999) 117–125.

[8] M. Kobayashi, N. Sada, M. Sugawara, K. Iseki, K. Miyazaki, Development of a new system for prediction of drug absorption that takes into account drug dissolution and pH change in the gastro-intestinal tract, Int. J. Pharm. 221 (2001) 87–94.

[9] X. He, M. Sugawara, M. Kobayashi, Y. Takekuma, K. Miyazaki, An *in vitro* system for prediction of oral absorption of relatively water-soluble drugs and ester prodrugs, Int. J. Pharm. 263 (2003) 35–44.

[10] X. He, S. Kadomura, Y. Takekuma, M. Sugawara, K. Miyazaki, A new system for the prediction of drug absorption using a pH-controlled Caco-2 model: evaluation of pH-dependent soluble drug absorption and pH-related changes in absorption, J. Pharm. Sci. 93 (2004) 71–77.

[11] X. He, M. Sugawara, Y. Takekuma, K. Miyazaki, Absorption of ester prodrugs in Caco-2 and Rat intestine models, Antimicrob. Agents Chemother. 48 (2004) 2604–2609.

[12] X. He, M. Sugawara, X.B. Zhu, S. Kadomura, Y. Takekuma, C.X. Liu, Application of an *in vitro* dissolution and absorption system to evaluate oral absorption of ketoprofen and two preparations of ketoprofen, AJPP 9 (2009) 203–210.

[13] M. Kataoka, Y. Masaoka, Y. Yamazaki, T. Sakane, H. Sezaki, S. Yamashita, *In vitro* system to evaluate oral absorption of poorly water-soluble drugs: simultaneous analysis on dissolution and permeation of drugs, Pharm. Res. 20 (10) (2003) 1674–1680.

[14] M. Kataoka, Y. Masaoka, S. Sakuma, S. Yamashita, Effect of food intake on the oral absorption of poorly water-soluble drugs: *in vitro* assessment of drug dissolution and permeation assay system, J. Pharm. Sci. 95 (9) (2006) 2051–2061.

[15] S.A. Motz, J. Klimundova, U.F. Schaefer, S. Balbach, T. Eichinger, P. Solich, C.M. Lehr, Automated measurement of permeation and dissolution of propranolol HCl tablets using sequential injection analysis, Anal. Chim. Acta 581 (2007) 174–180.

[16] S.A. Motz, U.F. Schaefer, S. Balbach, T. Eichinger, C.M. Lehr, Permeability assessment for solid oral drug formulations based on Caco-2 monolayer in combination with a flow through dissolution cell, Eur. J. Pharm. Biopharm. 66 (2007) 286–295.

[17] X. He, A Simulating Apparatus used for Evaluating Drug Complete Solid Dosage Forms, C.N. Patent, 201488990 U, 2009.

[18] Z.Q. Li, Z.D. Liu, C.C. Zhu, R. Liu, H. Juma, X. He, Evaluation on the release discipline of salvianolic acid B sustained-release tablets using a drug dissolution and absorption simulating system, Drug Eval. Res. 33 (5) (2010) 367–373.

[19] T. Taylor, L.F. Chasseaud, R. Major, E. Doyle, Isosorbide 5-mononitrate pharmacokinetics in humans, Biopharm. Drug Dispos. 2 (1981) 255–263.

[20] United States Pharmacopeial Convention, Dissolution, US Pharmacopeia 32-National Formulary 27, Rockville, MD, 1, 2009, pp. 711–720.

[21] R.W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, N.A. Peppas, Mechanisms of solute release from porous hydrophilic polymers, Int. J. Pharm. 15 (1983) 25–35.

[22] P.L. Ritger, N.A. Peppas, A simple equation for description of solute release II. Fickian and anomalous release from swellable devices, J. Control. Release 5 (1987) 37–42.

[23] United States Pharmacopeial Convention, *In vitro* and *In vivo* Evaluation of Dosage Forms, US Pharmacopeia 32-National Formulary 27, Rockville, MD, 1, 2009, pp. 1088–1089.

[24] National Pharmacopoeial Committee, Guidance for Sustained-release, Controlled Release and Delayed release Formulations, Pharmacopoeia of the People's Republic of China (Appendix XIX D), Beijing, 2, 2010, pp. 201–204.

[25] Y.B. Wang, J. Nedelman, Bias in the Wagner–Nelson estimate of the fraction of drug absorbed, Pharm. Res. 19 (4) (2002) 470–476.

[26] H. Akaike, Likelihood of a model and information criteria, J. Econom. 16 (1981) 3–14.

[27] Food and Drug Administration: Guidance for Industry: Statistical Approaches to Establishing Bioequivalence, US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research, 2001.

[28] P. Artursson, J. Karlsson, Correlation between oral drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial cells, Biochem. Biophys. Res. Commun. 175 (3) (1991) 880–885.

[29] R. Löbenberg, G.L. Amidon, Modern bioavailability, bioequivalence and biopharmaceutics classification system. New scientific approaches to international regulatory standards, Eur. J. Pharm. Biopharm. 50 (2000) 3–12.

[30] G. Corti, F. Maestrelli, M. Cirri, N. Zerrouk, P. Mura, Development and evaluation of an *in vitro* method for prediction of human drug absorption II, demonstration of the method suitability, Eur. J. Pharm. Sci. 27 (2006) 354–362.

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

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

[33] C.Y. Wu, L.Z. Benet, Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system, Pharm. Res. 22 (2005) 11–23.

[34] A. Dahan, J.M. Miller, G.L. Amidon, Prediction of solubility and permeability class membership: provisional BCS classification of the world's top oral drugs, AAPS J. 11 (4) (2009) 740–746.

[35] Food and Drug Administration: Guidance for Industry: Waiver of *In vivo* Bioavailability and Bioequivalence Studies for Immediate-release Solid Oral Dosage Forms based on a Biopharmaceutics Classification System, US Department of Health and Human Services/Food and Drug Administration/Center for Drug Evaluation and Research (CDER), 2000.

[36] O.A. Lake, M. Olling, D.M. Barends, *In vitro*/*in vivo* correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetics data obtained in healthy volunteers, Eur. J. Pharm. Biopharm. 48 (1999) 13–19.

[37] S. Li, H. He, L.J. Parthiban, H. Yin, T.M. Serajuddin, IV–IVC considerations in the development of immediate-release oral dosage form, J. Pharm. Sci. 94 (7) (2005) 1396–1417.

[38] P. Buch, P. Langguth, M. Kataoka, S. Yamashita, IVIVC in oral absorption for fenofibrate immediate release tablets using a dissolution/permeation system, J. Pharm. Sci. 98 (6) (2009) 2001–2009.

[39] A. Tangerman, A. Van Schaik, E.W. Van der Hoek, Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects, Clin. Chim. Acta 159 (1986) 123–132.

[40] T.L. Peeters, G. Vantrappen, J. Janssens, Bile acid output and the interdigestive migrating motor complex in normals and in cholecystectomy patients, Gastroenterology 79 (1980) 678–681.

[41] J.B. Dressman, G.L. Amidon, C. Reppas, V.P. Shah, Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms, Pharm. Res. 15 (1) (1998) 11–22.

[42] E. Galia, E. Nicolaides, D. Hörter, R. Löbenberg, C. Reppas, J.B. Dressman, Evaluation of various dissolution media for predicting *in vivo* performance of class I and II drugs, Pharm. Res. 15 (5) (1998) 68–75.

[43] S. Souliman, S. Blanquet, E. Beyssac, J.M. Cardot, A level A *in vitro*/*in vivo* correlation in fasted and fed states using different methods: applied to solid immediate release oral dosage form, Eur. J. Pharm. Sci. 27 (2006) 72–79.

[44] J.H. Hochman, M. Chiba, J. Nishime, M. Yamazaki, J.H. Lin, Influence of P-glycoprotein on the transport and metabolism of indinavir in Caco-2 cells expressing cytochrome P-450 3A4, J. Pharm. Exp. Ther. 292 (1) (2000) 310–318.

[45] C.L. Cummins, L.M. Mangravite, L.Z. Benet, Characterizing the expression of CYP3A4 and efflux transporters (P-gp, MRT1, and MRP2) in CYP3A4-transfected Caco-2 cells after induction with sodium butyrate and the phorbol ester 12-O-tetradecanoylphorbol-13-acetate, Pharm. Res. 18 (8) (2001) 1102–1109.