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Research Article

In Vitro-In Vivo Correlation for Solid Dispersion of a Poorly Water-Soluble Drug Efonidipine Hydrochloride

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Abstract. The aim of this present study was to investigate the ability of different dissolution methods to predict the *in vivo* performance of efonidipine hydrochloride (EFH). The solid dispersions of EFH were prepared by solvent evaporation method with HPMC-AS as matrix and urea as a pH adjusting agent. The paddle method, the open-loop, and the closed-loop flow-through cell methods were studied. In the study, Weibull's model was the best fit to explain release profiles. The pharmacokinetics behaviors of two kinds of solid dispersions with different release rate were investigated in comparison to the EFH after oral administration in rats. *In vivo* absorption was calculated by a numerical deconvolution method. In the study, the level A *in vivo* and *in vitro* correlation (IVIVC) was utilized. The correlation coefficient was calculated and interpreted by means of linear regression analysis (Origin.Pro.8.5 software). As a result, excellent IVIVC for solid dispersions and crude drug ($r^2 = 0.9352–0.9916$) was obtained for the dissolution rate determined with flow-through cell open-loop system in phosphate buffer solution with 0.1% (*w*/*v*) polysorbate 80 at pH 6.5, the flow-rate of 4 mL/min. In addition, the self-assembled flow cell system had good repeatability and accuracy. The dissolution rate of the solid dispersion could be slowed down by the flow-through method, and the difference caused by preparation was significantly distinguished. The study demonstrated that flow-through cell method of the open-loop, compared with paddle method, was suitable for predicting *in vivo* performance of EFH solid dispersions.

KEY WORDS: flow-through cell dissolution; *in vivo* and *in vitro* correlation; solid dispersion; efonidipine hydrochloride (EFH).

INTRODUCTION

Efonidipine hydrochloride (EFH) is a cyclophosphorylation-derived dihydropyridine calcium channel blocker developed by Nissan Chemical Industries, Ltd. (1,2). The currently marketed Landel® is a film-coated tablet prepared by a solid dispersion (3). The patent reported that the crude drug and hydroxypropyl methylcellulose succinate (HPMC-AS) were dissolved in an organic solvent mixed with ethanol and dichloromethane, and the solid dispersion was prepared by solvent evaporation method (4). Otsuka M et al. used HPMC-AS and urea to prepare a solid dispersion by microwave treatment to improve the poor solubility of EFH and improve the dissolution and apparent solubility (5).

Related studies have shown that the EFH tablets Landel® exhibits an enteric-soluble immediately release property. Moreover, the t_{max} of the 20 mg preparation in healthy adult male was about 1.7 ± 0.3 h (6). It was obvious that the dissolution rate in the paddle dissolution was too fast, the actual absorption in the body could not be reflected, and the IVIVC was not obtained. It has been reported that for immediate-release dosage form, the USP type IV dissolution apparatus is a better simulator of *in vivo* hydrodynamics than the paddle apparatus (7). Currently, a large number of studies of the *in vitro-in vivo* correlation comparing the paddle methods and the flow-through cell methods have been reported. In these studies, BCS class II drugs were the main research objects, with different dosage forms. Jinno JI. et al. successfully studied the IVIVC of a poorly water-soluble drug, cilostazol from the wet-milled tablet (8). Also Carol AM. et al. studied the fenofibrate-loaded mesoporous silica formulation (9), Mercuri A. et al. studied nifedipine immediate release capsules (10), and Medina JR. et al. studied carbamazepine immediate-release tablets (11) using the type IV dissolution apparatus. However, the *in vitro-in vivo* correlation studies of solid dispersions reported were basically paddle methods, and no meaningful IVIVC has been obtained in the reports (12,13). Therefore, *in vitro-in vivo* correlation of solid dispersion by comparing the paddle method and the flow cell method was studied. In this study, EFH was chosen as the model compound because of its low solubility. Solid dispersions were prepared by solvent evaporation to improve the dissolution rate of the EFH.

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Many *in vitro* dissolution experiments involving solid dispersion have utilized paddle method (9,12,14). There are limitations associated with this traditional dissolution approach with particular relevance to poorly water-soluble drug candidates (15). On the contrary, the type IV dissolution method has advantages for poorly water-soluble drugs. It can distinguish the effects of different preparations (16), processes (17), and different particle sizes of the preparation (18) on drug release. In addition, the method has the following advantages: simulating the gastrointestinal conditions in the body (19), flexibly changing the pH of the dissolution medium (20), and flexibly changing the volume of the dissolution medium according to the characteristics of the low-dose or large-dose preparations or the insoluble preparations (21).

In this study, the *in vitro* dissolution methods were studied to predict *in vivo* performance of solid dispersions. The paddle and self-made flow-through cell dissolution apparatus were employed to investigate the release of a poorly water-soluble drug, EFH, from solid dispersions. In addition, the selection of an appropriate dissolution media represents a critical step, when the common dissolution media are not able to dissolve those poorly water-soluble drugs in a quantitative manner (22). Therefore, the dissolution media containing the non-ionic surfactant, polysorbate 80 (tween 80), was also investigated. The pharmacokinetics behaviors of two kinds of solid dispersions with different release rate were investigated in comparison to the EFH after oral administration in rats. *In vivo* absorption was calculated by a numerical deconvolution method. In the study, the level A *in vivo* and *in vitro* correlation (IVIVC) was utilized.

MATERIALS AND METHODS

Materials

EFH was obtained from the Nissan Chemical Co., Ltd. (Tokyo, Japan). Nimodipine was supplied by Zhengzhou Ruikang Pharmaceutical Co., Ltd. (Zhengzhou, China). HPMC-AS was obtained from Shin-Etsu Chemical Ind. Co., Ltd. (Tokyo, Japan). Polysorbate 80 was obtained from Tianjin Hengxing Chemical Reagent Co., Ltd. (Tianjin, China). Urea was supplied by Aladdin Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical grade. Distilled water was used throughout the study.

Preparation of EFH Solid Dispersions

The solid dispersions of EFH were prepared by solvent evaporation method with HPMC-AS as matrix and urea as a pH adjusting agent. The ratios of EFH to HPMC-AS were shown in Table I. EFH and HPMC-AS were dissolved in dichloromethane and ethanol (1:1, *v*:*v*). If urea was added, the urea was first dissolved in ethanol and the remaining steps were consistent. The solvent was then evaporated in water bath at 50°C and dried under vacuum for 12 h. About 80% of the resultant solid dispersion was scraped out with a spatula. Solid dispersions were pulverized in a mortar and pestle and passed through 420 μm and 250 μm sieves before packing in an airtight container, stored in desiccators for further investigations.

Table I. The Composition of Solid Dispersion (SD) of EFH

Formula	EFH (g)	HPMCAS-LF (g)	Urea (g)
SD (1:1)	0.5	0.5	–
SD (1:3)	0.5	1.5	–
SD (1:4)	0.5	2.0	–
SD (1:3:1)	0.5	1.5	0.5

Characterization of EFH Solid Dispersions

Polarized Light Microscope. Samples were applied to a glass microscope slide and overlaid with a thin film of mineral oil. A DM2700P PLM apparatus (Leica Co., Germany) was used to confirm the amorphous nature of samples.

Powder X-Ray Diffraction. EFH solid dispersions (SDs) were spread on a graticulate frame and pressed by a slide. Patterns of samples obtained by diffractometer (DX-2700, Dandong Haoyuan Instrument Co., Ltd. China) and cu-kα line as a source of radiation, which was operated at the voltage 45 kV and the current 40 mA. All samples were measured in the 2θ angle range between 5° and 50° with a scanning range of 20°/min.

Solubility

The solubility of EFH in different concentration of polysorbate 80 solutions were determined at 37°C. Excess amount of EFH was added in 250 mL of the corresponding media in mini vessels. The paddle rotated at 200 rpm for 72 h. The suspensions were immediately filtered through a 0.22 μm nylon membrane filter, and the filtrate was diluted with an appropriate volume of methanol. Filtered solutions were analyzed for the EFH in UV spectrophotometer 330 nm.

Paddle Dissolution Test

According to the Chinese Pharmacopoeia (2015 Edition) for the dissolution and release method, the dissolution test was carried out, rotating at 50 or 100 rpm and a temperature of 37 ± 0.5°C (23). In addition, based on the previous solubility test results, 0.5% polysorbate 80 could meet the sink conditions. Solid dispersion equivalent to 20 mg of EFH was conducted in 250 mL phosphate buffer solution (10 mM) with 0.5% (*w*/*v*) polysorbate 80 at pH 6.5. Samples of dissolution media (5 mL) were withdrawn through a filter (0.22 μm) at each predetermined time, diluted ten times with blank dissolution media and assayed at 330 nm using UV-Visible spectrometer (UV-1800, Shimadzu) and replaced with fresh fluid. Dissolution studies were conducted in triplicate or sextuplicate.

Self-Made Flow-Through Cell Dissolution Test

Self-made flow-through cell dissolution tester was shown in Fig. 1. The open-loop pattern and the closed-loop pattern were shown in Fig. 2. The cell was made by the 10 mL syringe (20 mm i.d). The bottom of the syringe was filled with zirconia beads (2 mm i.d) to generate a laminar flow. The 200

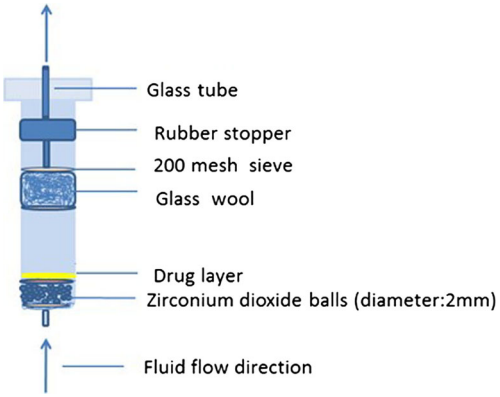

Fig. 1. Self-made flow-through cell

mesh sieve was placed on the top of the zirconia beads. The EFH was added on the sieve to ensure that the sample was always in the same position and did not come into contact with the zirconia beads. The top of the syringe was filter layer which could hold back the undissolved particles. The filter layer consisted of fiber glass wool (~ 200 mg) and covered with two 200 mesh sieves. The upper sieve contacted with a glass tube that passed through rubber stopper. The media used for the test were placed in a constant temperature water bath, maintained at 37 ± 0.5°C. Media delivery was achieved using a Precision Peristaltic Pump (BT100-2J, Lange Constant Flow Pump Co., Ltd., UK).

In the open-loop setting, the entire fluid passing through the cells for each sampling interval was collected. And the open-loop system was investigated using phosphate buffer (10 mM) with 0.5% (*w*/*v*) polysorbate 80 at pH 6.5 as a dissolution media at a flow-rate of 4 mL/min. In the closed-loop system, the dissolution media was circulating. And the close-loop setting was investigated using 250 mL of phosphate buffer (10 mM) with 0.5% (*w*/*v*) polysorbate 80 at pH 6.5 as a dissolution media at a flow-rate of 4 mL/min. The flow rate was 4, and the dissolution media in 1 h was 240 mL, which was close to the volume corresponding to the paddle method dissolution. Dissolution studies were conducted in triplicate or sextuplicate. The RSD of the accumulated release of the drug at different time points was within 5%. It is shown that the repeatability of the results of the dissolution process in the self-made flow-through cell was good. It could meet the detection requirements.

Pharmacokinetic Studies in Rats

Male Sprague-Dawley rats (weighing 200 ± 20 g) were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). All animal studies were approved by the Committee of Ethics of Animal Experimentation of Shenyang Pharmaceutical University. Twelve SD rats were randomly divided into four groups, with three in each group. The animals were allowed to acclimatize for 1 week before the experiment and were fed a standard diet and water *ad libitum*. Food was withdrawn 12 h prior to the study. Blood samples (0.3 mL) were collected at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h. All blood samples were collected in heparinized tubes and centrifuged immediately after withdrawal at 10000 rpm for 10 min at 4°C. Plasma samples were stored at − 60°C until analysis.

Bioanalytical Method

A 15 μL aliquot of nimodipine (40 μg/mL) as the internal standard (IS) and a 20 μL aliquot of NaOH (0.1 mol/L) were added to a 100 μL of plasma sample in a glass tube and vortexed for 30 s. The mixture was extracted with hexane–isopropanol (100:4, *v*/*v*) 3 mL, and centrifuged at 3000 rpm for 5 min. The supernatant was removed and evaporated to dryness at 40°C under a stream of nitrogen. The residue was reconstituted in 50 μL of mobile phase and 20 μL was injected into the column for HPLC analysis. All samples were analyzed by HPLC using a Shimadzu LC-10ATvp pump, a Shimadzu SPD-10Avp UV–VIS detector set at 254 nm, sensitivity setting of 0.01 absorbance units full scale (AUFS) with a Hypersil ODS C18 column (4.6 mm × 150 mm, 5 μm). The mobile phase consisted of acetonitrile and Milli-Q water (70:30, *v*/*v*) with a flow rate of 1.0 mL/min and an operating pressure of approximately 80 kgf/cm^2. All analyses were carried out at 35°C (24). Calibration curves (80–1200 ng/mL) and quality control (QC) samples (79.0, 395.2, and 1185.6 ng/mL) were prepared freshly for each analysis. The LLQQ by this method was 80 ng/mL. The recoveries of the QC samples ranged from 89.6 to 92.5%. The accuracy expressed by the relative standard deviation (RSD) were less than 15%. The recoveries of EFH in both were not less than 70%. The RSD of the intra- and inter-day precision were all less than 15%.

Pharmacokinetic Analysis

The maximum peak concentration of the drug in plasma (C_{max}), the time to reach the maximum concentration (t_{max}), and the area under the curve ($AUC_{0\text{-}24h}$) were obtained from plasma concentration-time curves. Absolute bioavailability (F_{abs}) was calculated according to Eq. 1.

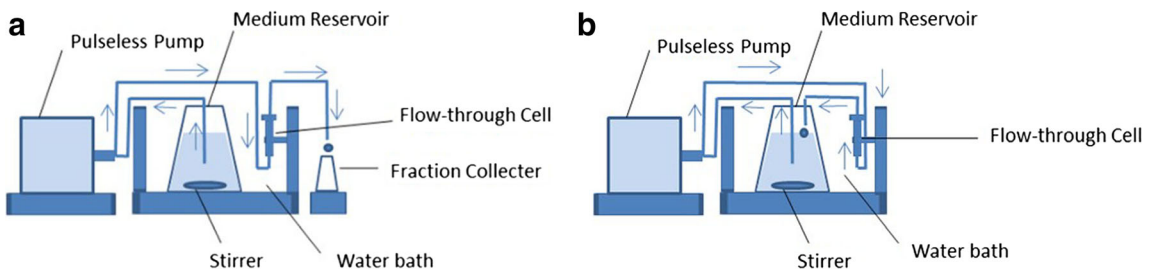

Fig. 2. Self-made flow-through cell dissolution systems: **a** open-loop and **b** closed-loop

$$F_{abs} = \frac{\mathrm{AUC}_{\mathrm{oral}}}{\mathrm{AUC}_{i.v.}} \times \frac{\mathrm{Dose}_{i.v.}}{\mathrm{Dose}_{\mathrm{oral}}} \times 100\% \quad (1)$$

where $\mathrm{AUC}_{\mathrm{oral}}$ and $\mathrm{Dose}_{\mathrm{oral}}$ are *AUC* and the dose for oral administration of EFH, respectively. $\mathrm{AUC}_{i.v.}$ and $\mathrm{Dose}_{i.v.}$ mean AUC and the dose for intravenous administration of EFH, respectively. The *in vivo* fraction absorbed of EFH was estimated using a numerical deconvolution method. The mean serum concentration data from the oral administration study were assigned as an output function (R), while the data from the intravenous administration study were used as a weight function (W), that is, the AUC in the equation. The AUC was calculated from 0 to i using a linear trapezoidal rule. According to the convolution-deconvolution theory, the input function (I) was calculated as follows Eq. 2.

$$I_i = \left[R_i - I_i \cdot \mathrm{AUC}_{t_i - t_1}^{t_i} - I_2 \cdot \mathrm{AUC}_{t_i - t_2}^{t_i - t_1} \cdots - I_{i-1} \cdot \mathrm{AUC}_{t_i - t_{i-1}}^{t_i - t_{i-2}}\right] / \mathrm{AUC}_0^{t_i - t_{i-1}} \quad (2)$$

Statistical Analysis

Standard pharmacokinetic (PK) parameters of EFH were obtained from plasma concentration-time curves using a non-compartmental model with DAS2.0 software. Data analysis was performed using the DDSolver program designed by Zhang Yong. Statistical analysis was performed by linear regression analysis using Origin.Pro.8.5 software. Further F tests were performed on the regression results, and the results were significantly correlated ($P < 0.05$).

RESULTS AND DISCUSSIONS

Preparation and Characterization of the EFH Solid Dispersions

Formula SD 1:1, SD 1:3, SD 1:4, SD 1:3:1 were prepared. The dissolution test was conducted in the close-loop system. Complete dissolution (> 90%) for SD 1:3:1 was achieved within 40 min, whereas complete dissolution (> 90%) for SD 1:3 and SD 1:4 were both achieved within 75 min. The dissolution test result showed that the dissolution rate of SD 1:1 was the lowest (complete dissolution (> 90%) within 120 min). As the content of HPMC-AS in the preparation increased, the dissolution rate of the drug increased. However, when the ratio of EFH to HPMC-AS was 1:4, the dissolution rate of the drug no longer increased, which was basically consistent with the dissolution rate of SD 1:3. After adding urea, the dissolution rate of SD 1:3:1 was higher than SD 1:3. In subsequent experiments, slow release SD 1:1 and rapid release SD 1:3:1 were chosen for the experiment. Preparation prescriptions were shown in Table I.

Polarized light microscopy analysis is the fastest way to examine whether the drug is in the amorphous state (25). The polarized light microscope images of solid dispersion are shown in Fig. 3. Birefringence existing in the crude drug (a) indicated that the bulk EFH was present in a massive crystalline form with a particle size of about 50 to 100 μm. A similar green spot of the crude drug was observed in the solid dispersion SD 1:1 (b). SD 1:3:1 (d) had dark green refracted light. Compared with blank control (f) which urea dispersed in HPMC-AS and urea (g). It could be inferred that the dark green light was a urea crystal form, and the crystal form state of the drug substance therein could not be judged, and other experimental verification was required.

X-ray power diffraction analysis was performed to determine the solid state of the drug in various formulations. The analysis of the PXRD results (Fig. 4) echoed the results of polarized light microscopy. The diffraction peak of the drug substance could be observed from the PXRD pattern, indicating a crystalline form. The solid dispersion SD 1:1 showed a characteristic diffraction peak of the crude drug, while the solid dispersion SD 1:3:1 did not show the diffraction peak. The diffraction peak appearing in SD 1:3:1 was the characteristic diffraction peak of urea. The above results confirmed that in the solid dispersion SD 1:1, part of the crude drug was present in a crystalline form, and in SD 1:3:1, the drug was present in an amorphous state.

Solubility of EFH in Different Concentrations of Polysorbate 80 Solutions

Equilibrium solubility of EFH in different concentrations of polysorbate 80 solutions were shown in Table II. It was confirmed that the solubility of EFH increased linearly with the increase of the concentration of polysorbate 80. A drug solubility of 0.185 mg/mL should be achieved to obtain sink conditions. Therefore, in the subsequent dissolution test, the 0.5% polysorbate 80 was selected as dissolution media.

Dissolution Studies with the Paddle Method

In the paddle method, the influence of different rotation speeds on the dissolution of the formulation was investigated. At first, *in vitro* dissolution studies were performed for the crude drug and solid dispersions at 100 rpm 250 mL with 0.5% polysorbate 80 solution. The obtained dissolution curves were illustrated in Fig. 5a. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 30 min and 10 min, respectively, whereas the accumulated release of crude drug was 44.7% ± 0.9% in 60 min. When the rotation speed was 75, the remaining conditions had not changed. The result was shown in Fig. 5b. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 30 min and 10 min, respectively, whereas the accumulated release of crude drug was 37.4% ± 1.8% in 60 min. When the rotation speed was 50, the remaining conditions had not changed. The result was shown in Fig. 5c. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 35 min and 10 min, respectively, whereas the accumulated release of crude drug was 20.0% ± 1.5% in 60 min. The above results indicated that all of solid dispersions improved the dissolution of crude drug. As the proportion of HPMC-AS increased, the formulation dissolved faster. But, the dissolution rate did not slow down as the rotation speed decreased. The dissolution profiles at three rotation speeds were almost identical. Compared with absorption *in vivo*, the dissolution *in vitro* was faster. All formulations were almost completely dissolved *in vitro* in 30 min. But The $t_{\max}$ of the crude drug, SD 1:1 and SD 1:3:1 were 5.0, 3.5, and 1.2 h, respectively.

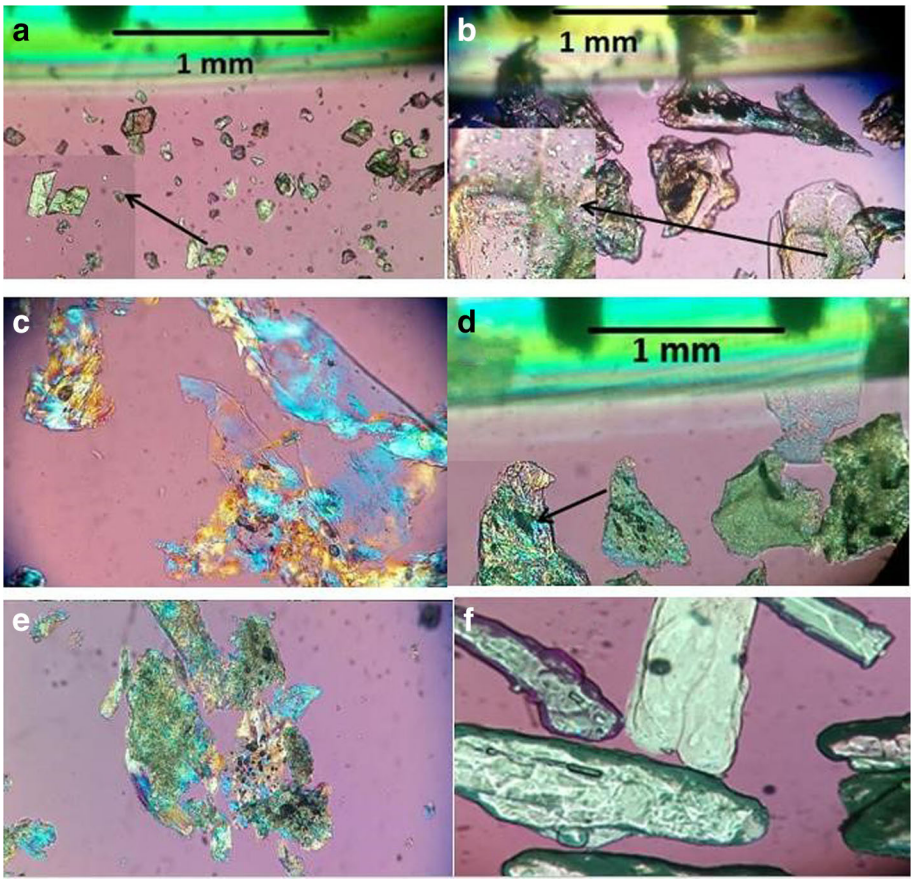

Fig. 3. Polarized light microscope images of solid dispersion and excipients: **a** crude drug, **b** SD 1:1, **c** HPMC-AS, **d** SD 1:3:1, **e** blank control (HPMC-AS + Urea), and **f** urea

Dissolution results of different formulations were not related to their absorption in rats.

Furthermore, the Conc. of polysorbate 80 as the dissolution media was studied. The rotation speed was 50, and the dissolution medium was pH 6.5 phosphate buffer solution, and the concentration of polysorbate 80 was 0.25%. The results were shown in Fig. 5d. The accumulated release of the EFH crude drug was 11.6 ± 1.6% in 60 min. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 40 min and 15 min, respectively. When the concentration of polysorbate 80 was 0.1%. The results were shown in Fig. 5e. The accumulated release of the EFH crude drug was 9.3 ± 0.4% in 60 min. For the solid dispersion SD 1:3:1 under this condition, the release was complete for 15 min. Subsequently, since the drug was in a supersaturated state under this condition, the dissolved drug crystallized and the detected value decreased. It was suspected that the hydrochloric acid in EFH was neutralized and became efonidipine. Since the solubility of efonidipine was lower than that of EFH (3), the drug was supersaturated to form crystals. Subsequently, the accumulated release rate again increased at 30 min of formula SD 1:3:1. It was guessed that the drug existed in the form of hydrochloride in the dissolution media at this time, so the solubility of the drug increased. And similar with formula SD 1:1. Complete dissolution (> 90%) for SD 1:3:1 was achieved within 40 min. It was found that even if the concentration of the surfactant polysorbate 80 was lowered, the dissolution rate of the solid dispersion could not be remarkably slowed, and the sensitivity of the detection could not be improved.

Dissolution Studies with Flow-Through Cell

Dissolution Studies with Flow-Through Cell of Close-Loop

As shown in the experimental section, flow-through cell could be applied in either "open-loop" (Fig. 2a) or "closed-loop" (Fig. 2b) setting. At first, the close-loop setting was investigated. The obtained dissolution curves were illustrated in Fig. 6a. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 90 min and 30 min, respectively, whereas the accumulated release of crude drug was 51.8 ± 3.1% in 180 min. It was obvious that the dissolution of the drug was slower than the paddle method.

Dissolution Studies with Flow-Through Cell of Open-Loop

The biggest difference between the open-loop system and the closed-loop system was that the media in contact with the drug was always fresh. The obtained dissolution curves by the open-loop were illustrated in Fig. 6b. Complete dissolution (> 90%) for SD 1:1 and SD 1:3:1 was achieved within 105 min and

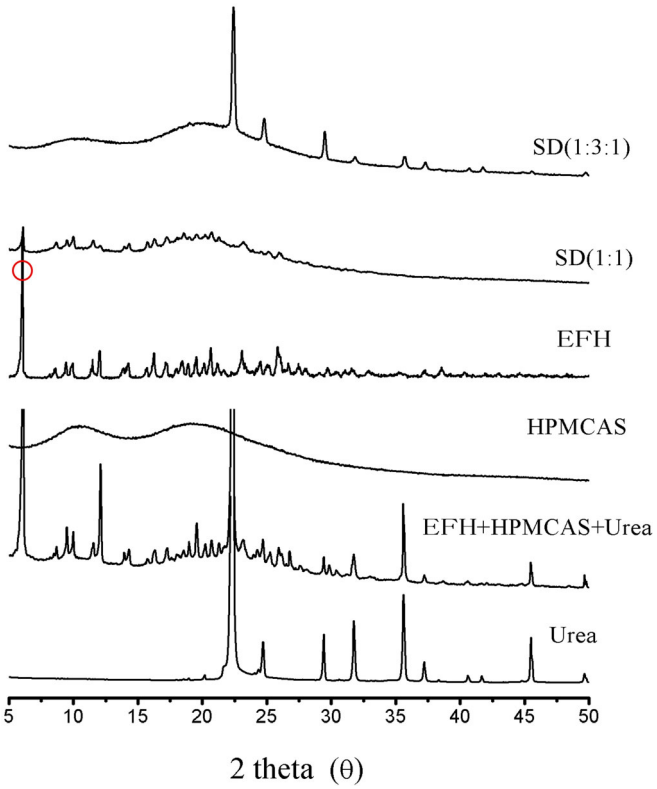

Fig. 4. XRPD patterns of different formulas of solid dispersion: SD 1:3:1, SD 1:1, crude EFH, HPMC-AS, physical mixture of EFH, HPMC-AS/urea and urea

40 min, respectively, whereas the accumulated release of crude drug was 53.3 ± 4.5% in 180 min. Like the closed-loop mode, its dissolution was also slowed down. In the open-loop mode, solid dispersion had no burst release, and the difference in dissolution of solid dispersions was clearly distinguished. For the crude drug, the drug concentration detected at different time points was basically unchanged. This shows that the drug substance was released at a constant rate. Further demonstrated that this process was zero-order release.

Since this experiment used a self-made flow cell, it was necessary to investigate the effects of factors such as flow rate on the experiment. Deirdre M. D'Arcy et al. (26) studied the effect of flow rate on dissolution experiments in flow-through method and found that the dissolution increased with increasing flow rate over the same time. The dissolution of the crude drug and the prescribed solid dispersion with different release rates in the open-loop mode at flow rates of 2, 4, and 8 mL/min were investigated. The results indicated that the flow rate had little effect on the poorly soluble drug substance and a large influence on the slow speed release prescription SD 1:1, and had little effect on the rapid release prescription SD 1:3:1. It could be seen that the release of the flow rate for dissolution of the formulation was primarily related to the release rate of the formulation itself. It had almost no effect on the rapid release preparation and had the greatest influence on the slow release preparation, and the dissolution increased as the flow rate increased. For the poorly soluble crude drug, the amount of dissolution increased as the flow rate increased within a certain flow rate range. When the flow rate was large, the fresh dissolution media was refreshed at a high rate, so that the drug was always under sink conditions, and the crude drug was released at a constant rate in the dissolution media (Fig. 7).

Table II. The Equilibrium Solubility of EFH at Different Concentration of Polysorbate 80 ($n = 3$)

Conc. of polysorbate 80 (%)	EFH (μg/mL)
0.05	20.5 ± 0.4
0.1	42.4 ± 2.9
0.2	83.6 ± 4.5
0.3	128.7 ± 7.5
0.4	154.1 ± 0.3
0.5	185.5 ± 4.8
0.6	213.7 ± 0.8

Furthermore, the Conc. of polysorbate 80 as the dissolution media was studied as with the paddle method. The results were shown in Fig. 8. For the rapid-release solid dispersion SD 1:3:1, the polysorbate 80 concentration were 0.5% and 0.25%, the dissolution curves were consistent ($f_2 >$ 50), and the dissolution rate became smaller at the same time in 0.1%. For the solid dispersion SD 1:1, the dissolution of the drug became smaller as the concentration of polysorbate 80 decreased during the same time, and the release of the drug was close to zero-order release at the lower polysorbate 80 concentration. For the crude drug, at the same time, as the concentration of polysorbate 80 decreased, the dissolution

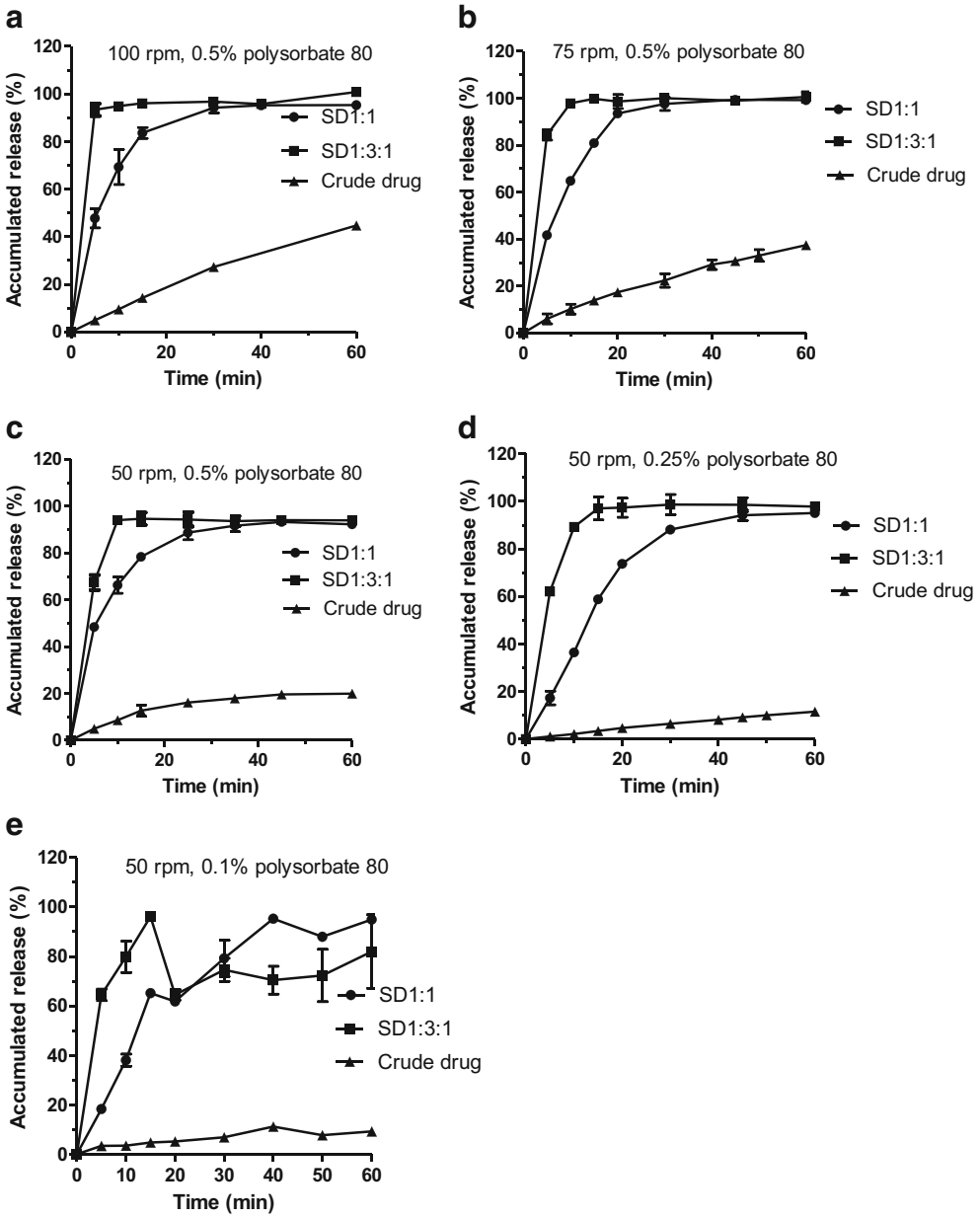

Fig. 5. Dissolution profiles of EFH from different solid dispersions by paddle method. **a** pH 6.5, 100 rpm, 0.5% polysorbate 80. **b** pH 6.5, 75 rpm, 0.5% polysorbate 80. **c** pH 6.5, 50 rpm, 0.5% polysorbate 80. **d** pH 6.5, 50 rpm, 0.25% polysorbate 80. **e** pH 6.5, 50 rpm, 0.1% polysorbate 80 (mean ± SD, $n = 3$)

rate became smaller, and the drug substance was released at zero level under different polysorbate 80 concentrations, and the release amount was positively correlated with the polysorbate 80 concentration in the same time.

Comparison of the Paddle, Flow-Through Cell Method of Close-Loop and Open-Loop

The dissolution results of the paddle method and flow-through cell method of close-loop and open-loop were shown in Fig. 9. In order to compare the similarity and difference of the dissolution curves of the same prescription in different dissolution methods, the dissolution curves of SD 1:3:1 in the paddle method were used as references. The dissolution curve f_2 factors of SD 1:3:1 in the closed-loop and open-loop modes of the flow-through cell were 38.09 and 40.28. In the paddle method, the solid dispersions dissolved too quickly without good discrimination. In contrast, the dissolution of solid dispersions slowed down using flow-through cell. All experiments were carried out under sink conditions. This indicated that hydrodynamic differences between the two model apparatus had a significant impact on the dissolution process.

To further understand the mechanism of the release of efonidipine hydrochloride from various formulations, data of *in vitro* release was fitted to different equations and kinetic models to explain release profiles. The kinetic models used were first-order equation, Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Makoid-Banakar, Weibull, and Logistic models (27). The largest correction coefficient (R^2_{adjusted})

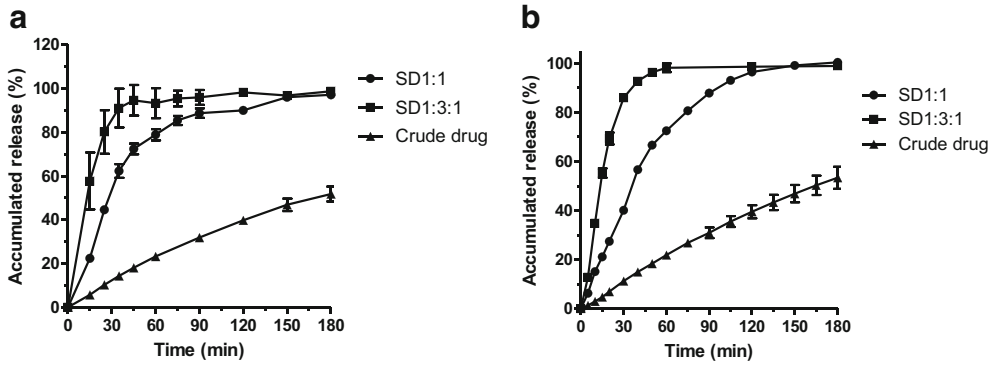

Fig. 6. Dissolution profiles of EFH from different solid dispersions by flow-through cell. **a** Closed-loop at 4 mL/min in 250 mL of phosphate buffer with 0.5% (*w*/*v*) tween 80 at pH 6.5. **b** Open-loop at 4 mL/min (mean ± SD, $n = 3$)

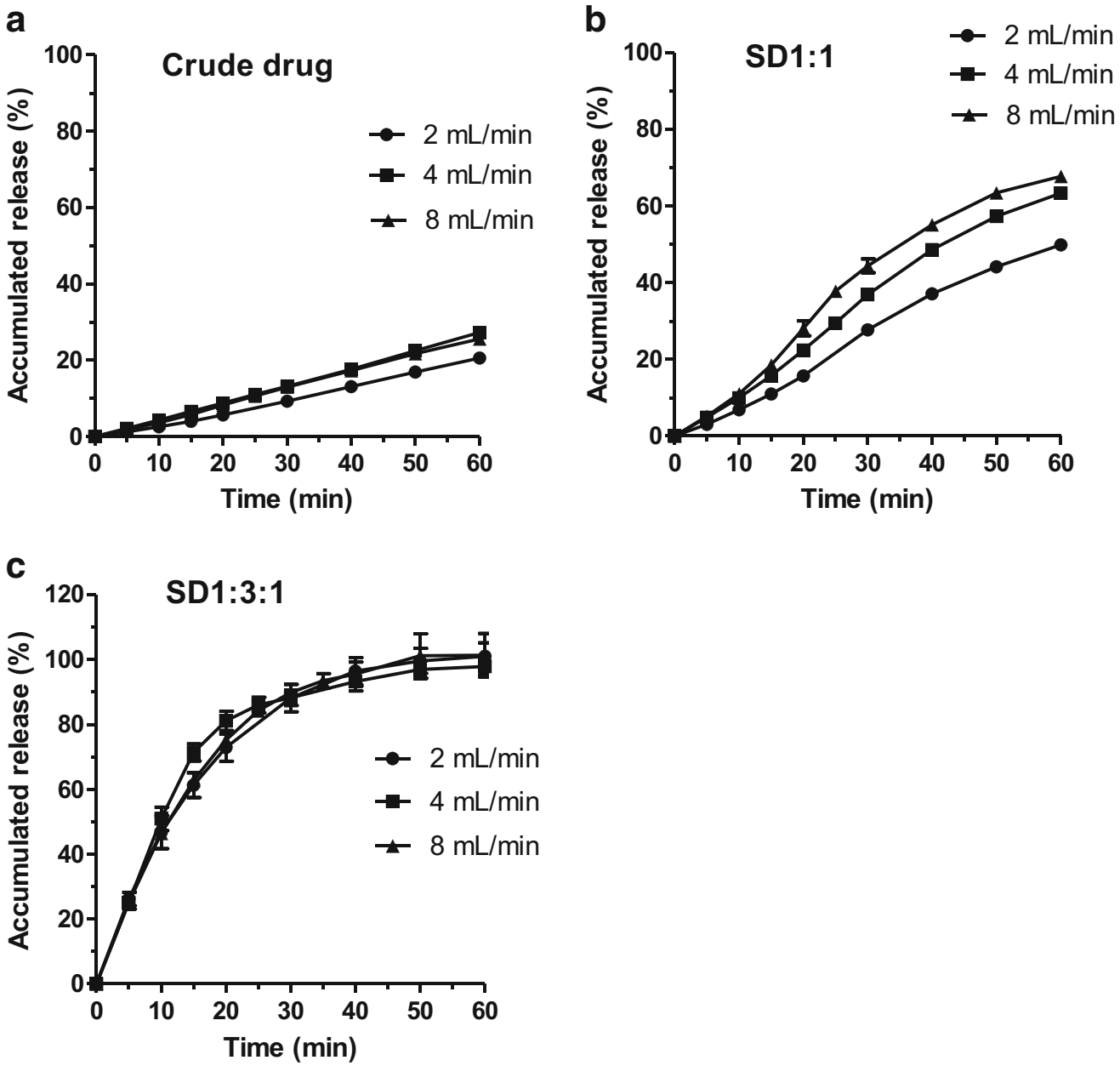

Fig. 7. The effect of flow rate on the dissolution of different formulas of solid dispersion at open-loop ($n = 3$)

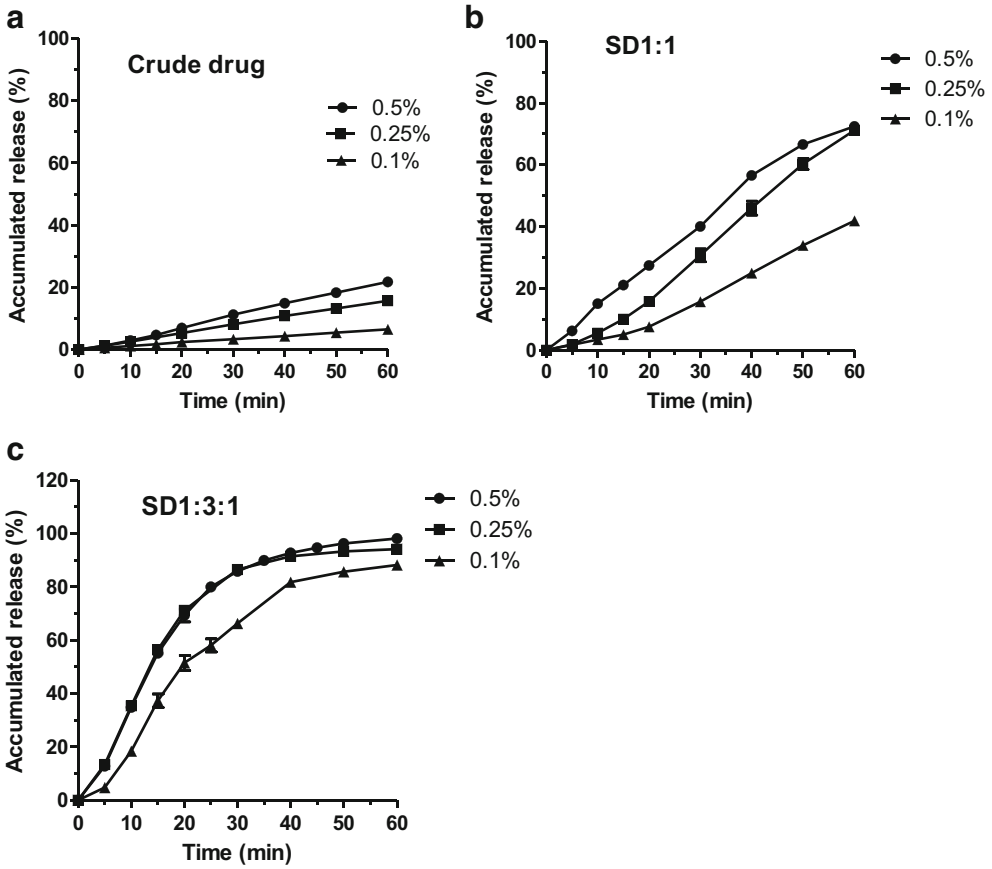

Fig. 8. The effect of polysorbate 80 concentration on the dissolution of different formulas of solid dispersion at open-loop ($n = 3$)

and the minimum Akaike's information parameter (AIC) in the model fitting were used as the basis for model selection (28). Data analysis was performed using the DDSolver program designed by Zhang Yong (29). The best fit (highest R^2 values and smallest AIC values) was observed in Weibull model, as shown in Table III. The formula for the Weibull equation was as follows Eq. 3.

$$F = F_{\max}\left[1-e^{-\frac{(t-T_i)^{\beta}}{\alpha}}\right] \tag{3}$$

where F is the release score corresponding to time t, F_{max} is the final release amount, α is the scale parameter, β is the shape parameter, and T_i is the position parameter. The difference in dissolution rate of the prescription was compared by T_{50} (time for 50% drug dissolve) and T_d (time for 63.2% drug dissolve). The results were shown in Table IV. According to the value of T_{50} and T_d, the open-loop and close-loop of the flow-through cell had greater advantage than the paddle method in distinguishing the differences between the formulations.

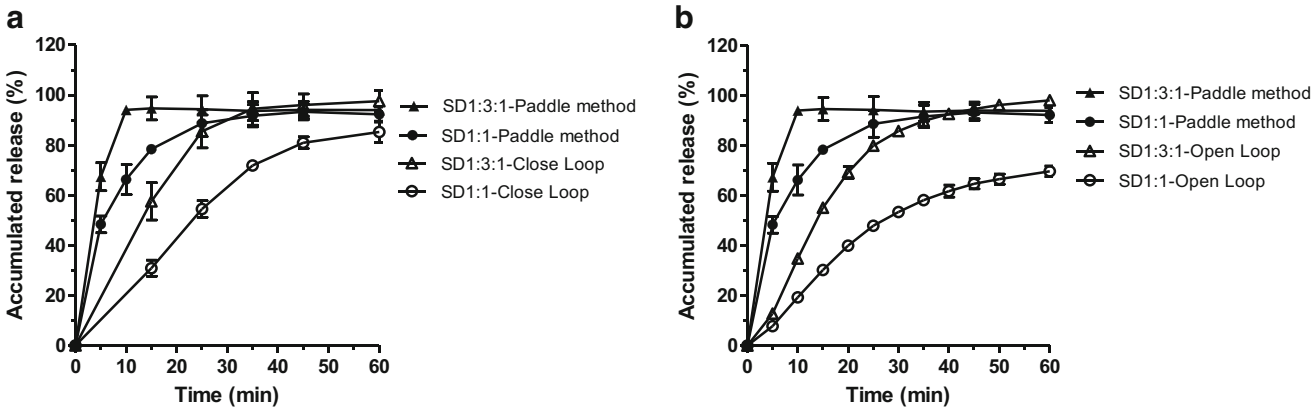

Fig. 9. The comparison of the paddle, flow-through cell of close-loop and open-loop ($n = 3$)

Table III. Criteria Used for the Selection of the Best Kinetic Model

Criteria	Method	Formula	First-order	Higuchi	Korsmeyer-Peppas	Hixson-Crowell	Makoid-Banakar	Weibull	Logistic
$R^2_{adjusted}$	Paddle	SD1:1	0.9872	0.6807	0.8491	0.8974	0.9933	0.9958	0.9885
		SD1:3:1	0.9135	0.1053	0.3629	0.3542	0.6256	0.9985	0.7554
		Crude drug	0.9944	0.9172	0.9224	0.8154	0.9920	0.9949	0.9350
	Close-loop	SD1:1	0.9495	0.9335	0.8916	0.9604	0.9997	0.9994	0.9945
		SD1:3:1	0.9268	0.6531	0.7337	0.9678	0.9577	0.9986	0.9972
		Crude drug	0.9908	0.9817	0.9889	0.9922	0.9991	0.9991	0.9947
	Open-loop	SD1:1	0.9855	0.9823	0.9442	0.9513	0.9982	0.9995	0.9932
		SD1:3:1	0.9674	0.9195	0.8732	0.9938	0.9896	0.9984	0.9975
		Crude drug	0.9991	0.9359	0.9998	0.9976	0.9999	0.9997	0.9976
AIC	Paddle	SD1:1	24.41	46.93	41.68	38.99	20.37	17.11	23.67
		SD1:3:1	30.53	46.88	44.50	44.60	41.22	2.37	37.80
		Crude drug	3.526	22.33	21.88	27.94	6.37	3.31	20.64
	Close-loop	SD1:1	25.60	26.98	29.42	24.39	0.57	3.39	14.54
		SD1:3:1	24.56	32.34	31.02	20.45	21.79	4.82	8.18
		Crude drug	5.33	8.75	6.286	4.47	− 6.25	− 6.06	2.61
	Open-loop	SD1:1	38.70	41.69	50.81	49.58	20.75	8.40	31.85
		SD1:3:1	51.77	61.09	63.98	36.78	42.07	25.19	28.56
		Crude drug	− 3.512	36.36	− 15.02	5.65	− 19.70	− 12.98	5.65

Pharmacokinetics

In this study, the concentrations of EFH in samples obtained from plasma of SD rats were determined by the method reported above. Figure 10 showed the plasma concentration *versus* time profiles and the pharmacokinetic parameters were listed in Table V. The crude drug provided only 7% bioavailability (*F*) of EFH. Solid dispersion SD 1:1 and SD 1:3:1 significantly increased the absorption, which exhibited in about 1.6-fold higher value of *F* (12.2%) and 1.8-fold higher value of *F* (13.7%), respectively. The maximum plasma concentration of 424.0 ± 148.2 ng/mL was observed for SD 1:3:1 formulation at 1.2 ± 0.8 h. Secondly, SD 1:1 formulation was slower with a C_{max} of 373.0 ± 92.8 ng/mL at t_{max} 3.5 ± 1.0 h. The crude drug of EFH was the slowest with a C_{max} 193.2 ± 24.8 ng/mL at 5.0 ± 2.6 h. A slower rate of drug absorption indicated a slower release profile. This indicated that the flow-through cell method better simulated how the solid dispersion formulation would perform *in vivo*. Further, in order to simulate *in vitro* and *in vivo* correlation, the fraction absorbed *versus* time profiles (Fig. 11) was calculated by a numerical deconvolution method.

Table IV. Weibull's T_{50} and T_d Values Derived from the Data Adjustment to This Kinetic Model

Method	Formula	T_{50}	T_d
Paddle	SD1:1	5.45	7.49
	SD1:3:1	4.08	4.56
Close-loop	SD1:1	22.71	24.93
	SD1:3:1	13.16	15.95
Open-loop	SD1:1	26.83	23.25
	SD1:3:1	13.88	17.24

In Vitro-In Vivo Correlation

The IVIVC model was divided into four levels: level A, level B, level C, and multiple level C. Level A IVIVC indicated a point-to-point correspondence between the *in vitro* release and the *in vivo* absorption. Usually, such correlations were linear, and in a linearly correlated condition, the *in vitro* dissolution directly coincided with the *in vivo* absorption curve or coincided by using a scaling factor. Level B IVIVC used the statistical moment principle to establish the correlation between the *in vitro* dissolution time average (MDTvitro) and the mean residence time (MRT) or the mean dissolution time *in vivo* (MDTvivo). Compared with the A-level, the B-level was not a point-to-point correlation. Therefore, relying only on the B-level correlation did not predict the actual blood concentration curve *in vivo*. Level C IVIVC was a single point correlation between a release point and a pharmacokinetic parameter, such as AUC, *c*, *t* etc. This correlation was a partial correlation, and the relevant parameters obtained could not reflect the shape of the blood concentration-time curve, nor the whole release process and the characteristics of the whole absorption process. Multiple level C constructed a multi-point correlation between one or several relevant pharmacokinetic parameters and drug dissolution at different time points *in vitro* dissolution test. Multiple C-level IVIVC included at least three time-point drug release characteristics, and the selected time points should reflect the dissolution characteristics of early, middle, and late stages (30–33). In our study, the level A IVIVC was utilized. *In vitro* and *in vivo* results were taken as independent (*x*) and dependent (*y*) variables, respectively. The correlation coefficient was calculated and interpreted by mean of linear regression analysis (Origin.Pro.8.5 software).

Figure 12 showed the correlation between the *in vitro* dissolved fractions obtained by flow-through cell open-loop system and close-loop system (4 mL/min), and the fractions absorbed in the rats. In addition, the correlation between *in vitro* dissolved fractions obtained by paddle method and

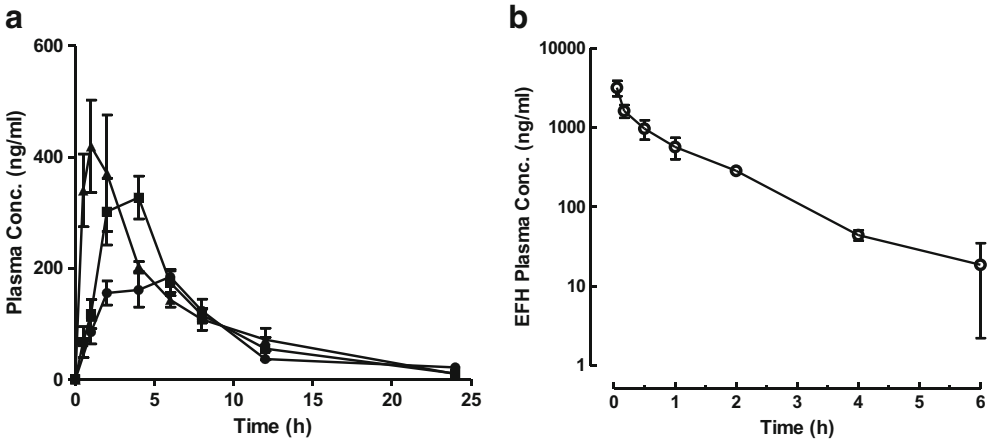

Fig. 10. Plasma concentration of EFH *vs.* time profiles after oral administration of the equivalent of 10 mg/kg EFH to fasted rats (**a**), (black circle) crude drug, (black square) SD 1:1 formulation, (black triangle) SD 1:3:1 formulation. Plasma levels of EFH in rats (white circle, 1 mg/kg) after intravenous administration (**b**) (mean ± SD, $n = 3$)

the fractions absorbed in the rats were also in Fig. 12. The dissolution media of the three dissolution methods was phosphate buffer solution with 0.5% (*w*/*v*) tween 80 at pH 6.5. According to the deconvolution analysis, the absorption in rats was completed.

Excellent correlations were found for crude drug ($R^2 = 0.9798$, $P < 0.001$), SD 1:1 ($R^2 = 0.9193$, $P < 0.01$), and SD 1:3:1 ($R^2 = 0.9008$, $P < 0.05$) *in vitro* dissolution and *in vivo* absorption in open-loop system. Second, better correlations were found for crude drug ($R^2 = 0.9758$, $P < 0.01$), SD 1:1 ($R^2 = 0.6772$, $P > 0.1$), and SD 1:3:1 ($R^2 = 0.8730$, $P < 0.05$) *in vitro* dissolution and *in vivo* absorption in close-loop system. The worst correlations were found for SD 1:1 and SD 1:3:1 *in vitro* dissolution and *in vivo* absorption in paddle method ($R^2 = 0.4533$, $P > 0.1$ and 0.0668, $P > 0.1$). However, the crude drug had a good correlation *in vitro* dissolution and *in vivo* absorption in paddle method ($R^2 = 0.9823$, $P < 0.001$). The above results indicated that it had good *in vitro* and *in vivo* correlation regardless of the dissolution method for the crude drug. However, for solid dispersion formulations, the flow cell dissolution method presented a better advantage in terms of *in vitro* and *in vivo* correlation than the paddle method. Although *in vitro* dissolution was not completely consistent with *in vivo* absorption, it had a high correlation from the experimental results. It might be caused by the complexity of the internal environment. Various gastrointestinal (GI) factors affect drug and formulation behavior after oral administration, including GI transfer, motility, pH and GI fluid volume, and composition (34). Although the flow cell was closer to the body in terms of fluid mechanics than the paddle method, it did not completely replicate the environment of the intestine.

IVIVCs for different polysorbate 80 concentrations were also studied. Figures 13 and 14 showed the correlation between the *in vitro* dissolved fractions obtained by paddle method or flow-through cell method open-loop system and the fractions absorbed in the rats with different polysorbate 80 concentrations (0.5%, 0.25%, 0.1%). According to the deconvolution analysis, the absorption in rats was completed.

In paddle method, better IVIVC were found for crude drug in 0.5% polysorbate 80 ($R^2 = 0.9823$, $P < 0.001$) and 0.25% polysorbate 80 ($R^2 = 0.9752$, $P < 0.01$). The worst IVIVC was found for crude drug in 0.1% polysorbate 80 ($R^2 = 0.6423$, $P < 0.05$), which may be related to drug supersaturating. For SD 1:1, poor IVIVCs were found in 0.5% polysorbate 80 ($R^2 = 0.4533$, $P > 0.1$), 0.25% polysorbate 80 ($R^2 = 0.6426$, $P < 0.05$), and 0.1% polysorbate 80 ($R^2 = 0.7020$, $P < 0.01$). However, the IVIVC improved as the concentration of polysorbate 80 decreased. For SD 1:3:1, poor IVIVCs were found in 0.5% polysorbate 80 ($R^2 = 0.0668$, $P > 0.1$), 0.25% polysorbate 80 ($R^2 = 0.4405$, $P > 0.1$), and 0.1% polysorbate 80 ($R^2 = 0.0467$, $P > 0.1$).

Table V. Pharmacokinetic Parameters of Different Formulas In Rat Plasma After Orally Administrated at a Dose of 10 mg/kg ($n = 3$)

Parameters	Formula		
	Crude drug	SD 1:1	SD 1:3:1
$t_{1/2}$ / h	4.2 ± 1.2	4.5 ± 1.6	3.8 ± 0.7
t_{max} / h	5.0 ± 2.6	3.5 ± 1.0	1.2 ± 0.8
C_{max} / μg/L	193.2 ± 24.8	373.0 ± 92.8	424.0 ± 148.2
$AUC_{(0-24)}$ /(μg/L h)	1455.6 ± 132.1	1975.8 ± 498.1	2203.9 ± 322.1
$AUC_{(0-\infty)}$ /(μg/L h)	1672.9 ± 92.7	2607.2 ± 331.3	2925.5 ± 854.3

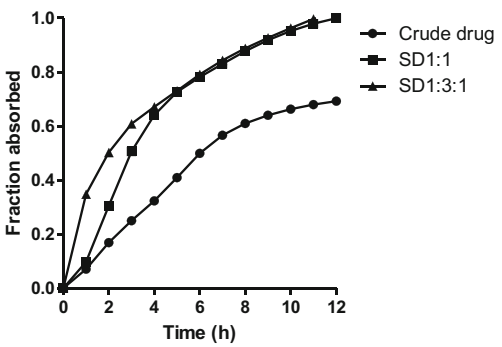

Fig. 11. Fraction absorbed-time profiles of EFH after oral administration of crude drug, SD 1:1, and SD 1:3:1

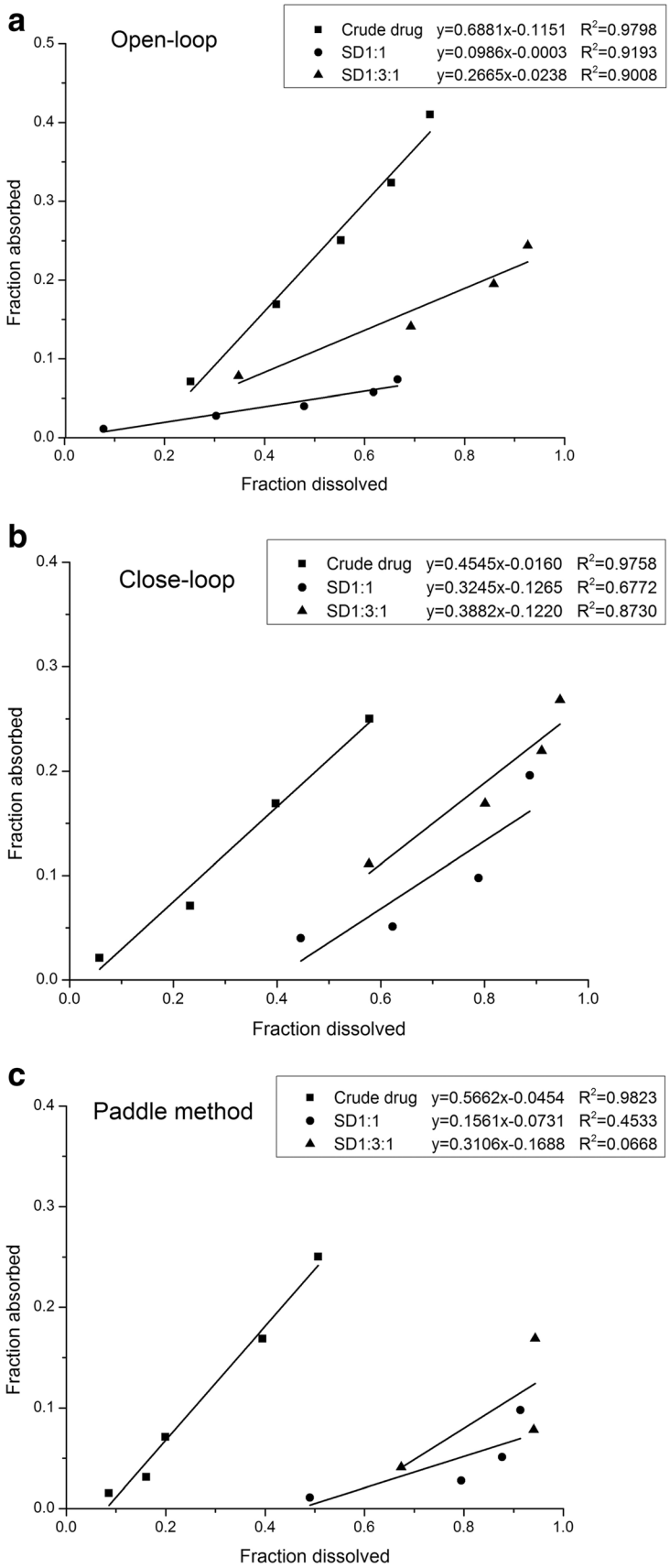

Fig. 12. *In vivo* and *in vitro* relation of crude drug, SD 1:1, and SD 1:3:1 in different dissolution methods

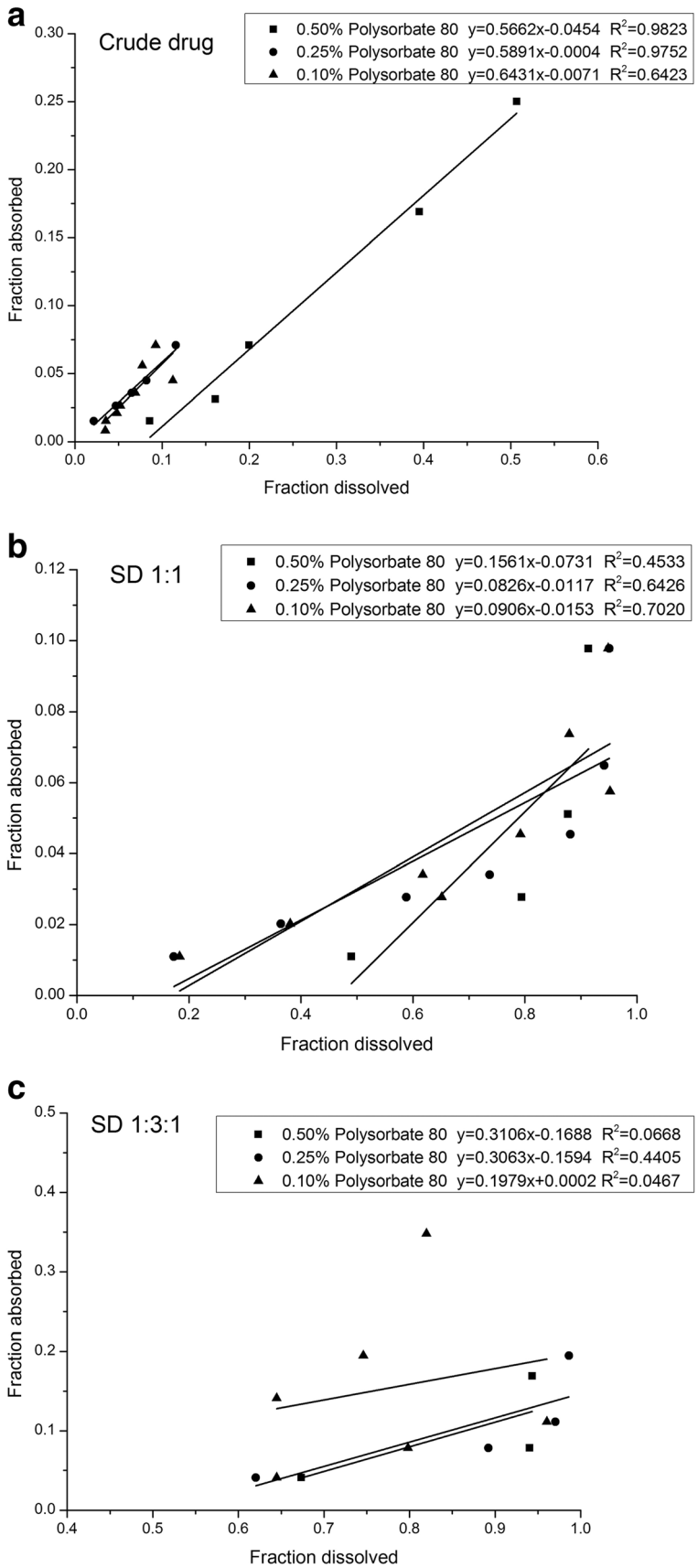

Fig. 13. *In vivo* and *in vitro* relation of crude drug, SD 1:1, and SD 1:3:1 in paddle method with different polysorbate 80 concentrations

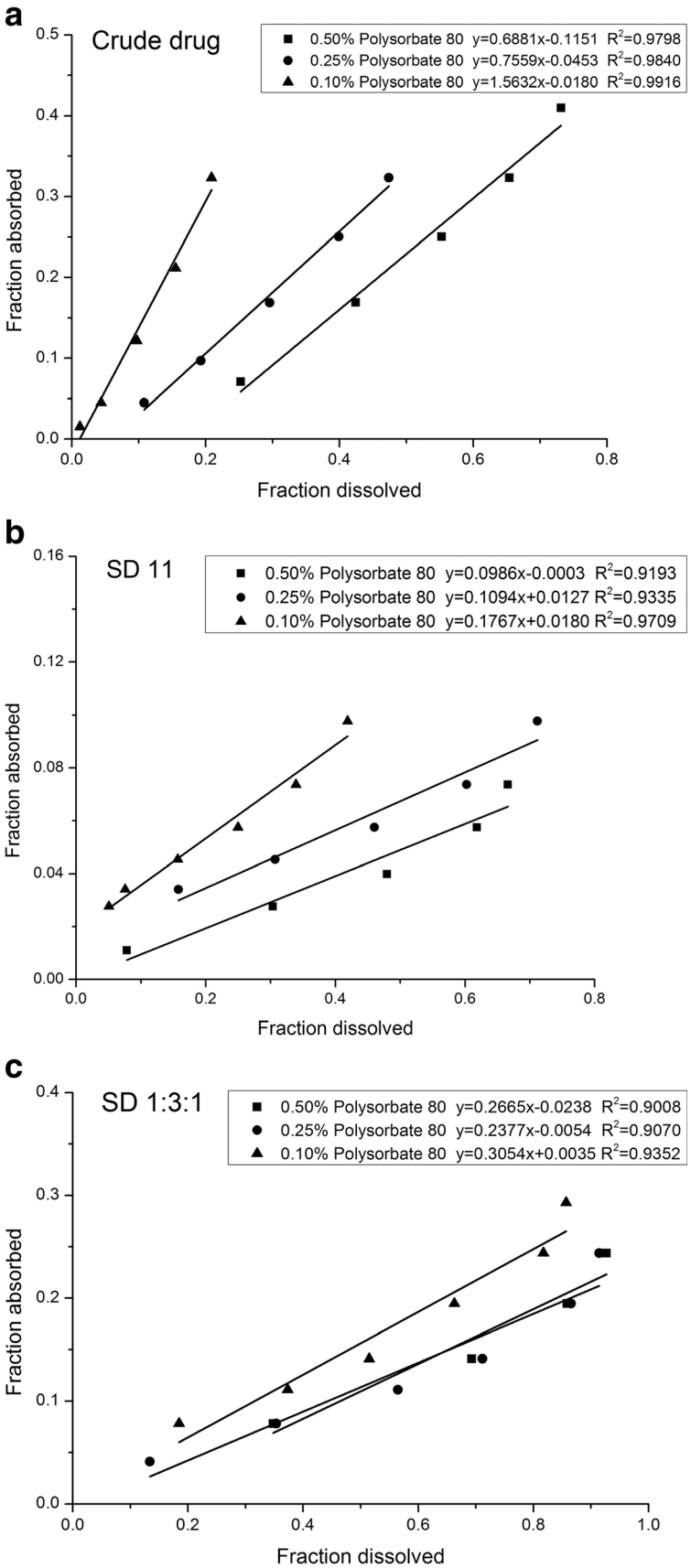

Fig. 14. *In vivo* and *in vitro* relation of crude drug, SD 1:1, and SD 1:3:1 in flow-through cell open-loop system with different polysorbate 80 concentrations

In flow-through cell method open-loop system, excellent IVIVCs were found for crude drug ($R^2 = 0.9916$, $P < 0.001$), SD 1:1 ($R^2 = 0.9709$, $P < 0.001$), and SD 1:3:1 ($R^2 = 0.9352$, $P < 0.01$) were found in 0.1% polysorbate 80. Better IVIVCs were found for crude drug ($R^2 = 0.9840$, $P < 0.001$), SD 1:1 ($R^2 = 0.9335$, $P < 0.01$), and SD 1:3:1 ($R^2 = 0.9070$, $P < 0.01$) were found in 0.25% polysorbate 80. Good IVIVCs were found for crude drug ($R^2 = 0.9798$, $P < 0.001$), SD 1:1 ($R^2 = 0.9193$, $P < 0.01$), and SD 1:3:1 ($R^2 = 0.9008$, $P < 0.05$) were found in 0.1% polysorbate 80.

According to the above research data of *in vitro* and *in-vivo* correlations, it was known that all formula had better IVIVCs in the open-loop mode of the flow-through cell. And in this mode, all formula had excellent IVIVCs in the dissolution media containing 0.1% polysorbate 80. Tang et al. also obtained better IVIVC of a new chemical entity in various formulations under non-sink conditions (35). The reasons that the flow-through cell method had better *in vitro-in vivo* correlation were as follows: the flow-through cell method could replenish a fixed volume of dissolution media at a certain rate, thereby simulating the liquid flow in the digestive tract. In addition, the zirconia beads at the bottom of the flow-through cell could change the flow of the dissolution media, and at the same time could simulate the friction of the digestive tract (36). The flow-through cell had no agitation mechanisms exist and the dosage form and the drug particles were continuously exposed to a uniform laminar flow, similar to the natural environment of the gastrointestinal tract (11).

CONCLUSIONS

The solid dispersion of EFH and HPMC-AS was prepared by solvent evaporation method. Pharmacokinetic experiments showed that the rapid release of the prescription SD 1:3:1 and the slow release of the prescription SD 1:1 improved the absorption of the EFH drug substance in rats. However, there were some differences in the rate and extent of absorption, which corresponded with the results of dissolution *in vitro*. Under the dissolution of the paddle method, the dissolution rate of all the preparations was too fast. It did not correlate well with the absorption *in vivo*. A reasonable IVIVC ($r^2 = 0.9352$–0.9916) was obtained for the dissolution rate determined with flow-through cell open-loop system in phosphate buffer solution with 0.1% (*w*/*v*) polysorbate 80 at pH 6.5, the flow-rate of 4 mL/min. The self-assembled flow cell system had good repeatability and could meet the requirements of the experiment. Under the flow cell dissolution method, the sensitivity was high, the dissolution rate of the solid dispersion in each formulation could be slowed down, and the difference in dissolution caused by prescription differences was significantly distinguished.

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