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Brief/Technical Note

Miniaturized Transfer Models to Predict the Precipitation of Poorly Soluble Weak Bases upon Entry into the Small Intestine

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Abstract. For poorly soluble weak bases, the possibility of drug precipitation upon entry into the small intestine may affect the amount of drug available for uptake through the intestinal mucosa. A few years ago, a transfer model was introduced which has been developed to simulate the transfer of a dissolved drug out of the stomach into the small intestine. However, this setup requires the use of clinically relevant doses of the drug, which are typically not available in the early stages of formulation development. The present series of tests was performed to check whether it is possible to create a miniaturized but physiologically relevant transfer model that can be applied in the early formulation development. Experiments were performed with two miniaturized setups: a 96-well plate model and a mini-paddle transfer system. Itraconazole and tamoxifen were used as model drugs. An appropriate amount of each drug formulation was dissolved in simulated gastric fluid and then transferred into an acceptor phase consisting of fasted/fed state simulated small intestinal fluid. The amount of drug dissolved in the acceptor phase was monitored over a period of 4 h. Results from both setups were very similar. The tamoxifen preformulation did not precipitate, whereas the itraconazole formulation precipitated to the same extent in both setups. Due to the possibility of generating physiologically relevant results but using smaller sample sizes and smaller volumes of media, both miniaturized transfer systems offer various advantages in terms of substance and analytical and material cost savings when evaluating the precipitation potential of poorly soluble weakly basic drug candidates.

KEY WORDS: 96-well plate; biorelevant media; drug precipitation; gastric emptying; transfer model.

INTRODUCTION

Precipitation of orally administered drugs in the gastrointestinal (GI) tract is an undesirable process which often can be observed upon the entry of solutes containing poorly soluble weak bases into the small intestine. Precipitation can be a result of a sharp pH change, the dilution of the formulation with GI fluids, or the digestion of solubilizing agents in the formulations (1).

For poorly soluble weak bases, precipitation in the small intestine can reduce the amount of drug available for absorption and, thus, can affect systemic exposure. Therefore, to ensure both safety and efficacy after oral administration of such compounds, it is equally important to design formulations that help to overcome solubility and precipitation issues and to be able to assess the drug precipitation potential in an *in vitro* setup in early formulation screening.

In the pharmaceutical industry, various high-throughput methods are applied to determine the kinetic or thermodynamic (equilibrium) solubility of drug candidates. Kinetic solubility is often determined by first dissolving the drug in a polar aprotic organic solvent such as dimethyl sulfoxide and then adding this drug solution to a buffer in a 96-well plate. The solution is then allowed to equilibrate for a predetermined time in which the drug may precipitate. After filtration, the drug remaining in solution is measured using an UV plate reader (2). This kinetic solubility approach provides for a rapid result, but even when using buffers of physiological pH values, the results are altered by the presence of the organic solvent and, thus, the solubility is often overestimated (3). Therefore, such methodologies are useful for an initial screening, but their physiological relevance is rather small. Recently, some—with respect to their biorelevance—more advanced screening methods using 96-well plate setups were described in the literature (3–5). However, these methods either focused only on determining the equilibrium solubility in biorelevant media or were not designed to simulate the entry of drug solutes containing poorly soluble weak bases into the small intestine in all relevant details.

A few years ago, a transfer model simulating the transfer of a dissolved drug out of the stomach into the small intestine and also addressing different prandial states (6) was introduced. In comparison to high-throughput methods, this model seems to be more predictive for the *in vivo* situation. However, it had been

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Table I. Test Doses and Media Volumes Required for a Transfer Experiment in a Single Well in the 96-Well Plate Model and a Single Vessel in the Mini-Paddle Transfer Model

Setup	Test dose	Volume donor phase	Volume acceptor phase	Total volume	Theoretical drug conc. after transfer (μg/mL)
Mini-paddle transfer system	100 mg itraconazole	40 mL	225 mL	265 mL	377.5
	10 mg tamoxifen				37.75
96-Well plate model	75.5 μg itraconazole per well[a]	30 μL	170 μL	200 μL	377.5
	7.55 μg tamoxifen per well[a]				37.75

[a] Before starting the experiments, stock solutions of 5.6625 mg itraconazole in 15 mL SGFsp, pH 1.2, and 1.1325 mg tamoxifen in 30 mL SGFsp, pH 1.2, were prepared to obtain the donor phase

developed to work with physiological volumes of media and requires the use of clinically relevant doses of the drug, which are often not available in early formulation screening. Thus, a miniaturized transfer model would be advantageous, even at the earliest stages of development where clinically relevant doses of the drug are unknown and the aim is to better understand the risk of concentration-dependent intestinal drug precipitation of poorly soluble weakly basic compounds.

The present series of tests was performed to check whether it is possible to create a miniaturized but still physiologically relevant transfer model that can be used as a screening tool for formulations containing poorly soluble weak bases.

MATERIALS AND METHODS

Materials

Drug substances were purchased from Sequoia Research Products, Pangbourne, UK. Sodium taurocholate, batch no. 2002040113 was purchased from Prodotti Chimici Alimentari S.P.A., Basaluzzo (AL), Italy. Egg lecithin, Lipoid EPCS, batch no. 105026-1, was kindly donated by Lipoid GmbH, Ludwigshafen, Germany. All other compounds were of analytical grade and purchased commercially.

Experimental Setup

Experiments were performed with two miniaturized setups: a 96-well plate model and a mini-paddle (7) transfer system. Two poorly soluble weak bases, namely, tamoxifen-free base (pK_a=8.8) and itraconazole (pK_a=approx. 3.7) were used as model drugs. Experiments were performed in the course of screening the *in vitro* performance of hydroxybutenyl-β-cyclodextrin (HBenBCD) complexes of these compounds (8,9) and were designed to simulate the oral administration of a single dose of 200 mg itraconazole or 20 mg tamoxifen, respectively. HBenBCD complexes of the drugs were used since, particularly with itraconazole, it would not have been possible to dissolve a single dose in simulated gastric medium because of its very poor solubility even in acidic media. Modified fasted state simulating intestinal fluid (FaSSIF, pH 7.5) and fed state simulating intestinal fluid (FeSSIF, pH 5.0) were used as acceptor media (10,11). These media were chosen to represent physicochemical parameters (particularly pH, osmolality, and the presence of bile compounds) typical for the fasted and the fed state small intestine after transferring a fixed volume of simulated gastric fluid without pepsin (SGFsp, pH 1.2) into the acceptor medium. The amount of drug and the volumes of the media chosen for both sets of experiments represent a scale down of the physiological relevant doses and volumes used in the "regular-scale" transfer model (6). In the model applied by Kostewicz *et al.*, SGFsp with a pH of ~2 and FaSSIF, pH 6.5, were used as the donor and acceptor phases, respectively. In contrast, in the present series of experiments, we had to use a SGFsp, pH 1.2, to ensure complete drug dissolution under gastric conditions and thus decided to use a pH-modified FaSSIF with a pH of 7.5 as the acceptor phase. The reason for this decision was to obtain a pH of 6.5, i.e., a realistic pH in the upper/mid small intestine after complete transfer of the gastric drug solution. With the relatively low buffer capacity of FaSSIF, pH 6.5, this would have not been

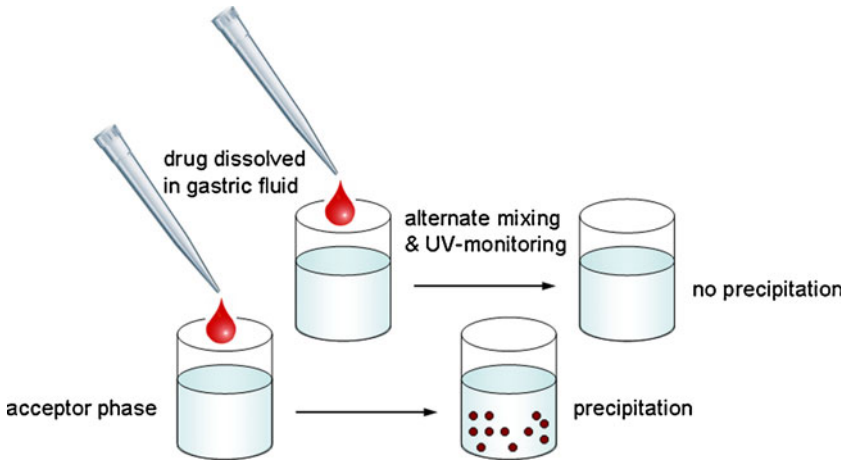

Fig. 1. Experimental setup for the 96-well plate transfer experiments (donor phase transfer)

Table II. Experimental Setup for the 96-Well Transfer Experiments (Plate)

Row	Well	Volume (μL)	Acceptor phase	Vol. added (μL)	Donor phase
A	1-12	170	FaSSIF, pH 7.5	30	Drug in SGFsp, pH 1.2
B	1-12	170	FeSSIF, pH 5.0	30	Drug in SGFsp, pH 1.2
C	1-12	170	FaSSIF, pH 7.5	30	Drug in SGFsp, pH 1.2
D	1-12	170	FeSSIF, pH 5.0	30	Drug in SGFsp, pH 1.2
E	1-12	170	SGFsp, pH 1.2	30	Drug in SGFsp, pH 1.2
F	1-12	170	FaSSIF, pH 7.5	30	SGFsp, pH 1.2
G	1-12	170	FeSSIF, pH 5.0	30	SGFsp, pH 1.2

possible since the resulting pH was measured to be pH ~3. As the neutralizing capacity of the duodenum is relatively high and, with a normal pancreatic function *in vivo*, the average duodenal/jejunal pH range is rapidly attained (12), a pH of ~3 would not be representative for our purpose. Moreover, such a low pH could contribute to the presence of the ionized and more soluble form of the drug and, thus, affect precipitation. Therefore, we had performed preliminary experiments to screen for a simulated fasted intestinal medium that still had a biorelevant pH, but did not come along with such a sharp decrease in pH when the acidic drug solution was added. Taking into account the volumes that we intended to transfer, FaSSIF, pH 7.5, prepared by simply increasing the sodium hydroxide content of the original FaSSIF medium, appeared to be adequate for our purpose. Using FaSSIF, pH 7.5, as the acceptor phase, we obtained a pH of 6.5 after complete transfer and we could also get an idea what would happen if the dissolved drug would get into contact with fluids of neutral or slightly alkaline pH, e.g., pancreatic fluid (13). Since FeSSIF, pH 5.0, has a much higher buffer capacity than FaSSIF, pH 6.5, for the fed state experiments, there was no need to modify the acceptor medium since even after transferring the calculated amount of drug dissolved in SGF, pH 1.2, no significant pH change of the acceptor phase (FeSSIF, pH 5.0) could be observed.

96-Well Plate Model

The first set of tests was performed in a 96-well plate. The temperature was 37°C and UV-Star® flat-bottom 96-well plates (Greiner Bio-One, Monroe, NC, USA) were used in all experiments. First, an appropriate amount of the drug formulation (see Table I) was completely dissolved in SGFsp, pH 1.2 (donor phase). Then, a multichannel microliter pipette was used to transfer 30 μL of the donor phase into wells containing 170 μL of the acceptor phase represented by either pH-modified FaSSIF, pH 7.5, or FeSSIF, pH 5.0 (see Fig. 1). 2×12 wells ("transfer wells") were used for each acceptor medium. In addition, in another 12 wells, we added 30 μL of the donor phase to 170 μL SGFsp, pH 1.2, to obtain a reference solution with 100% of the dose being dissolved (377.5 μg/mL itraconazole and 37.75 μg/mL tamoxifen). Finally, in other 2×12 wells ("blind wells"), we added 30 μL SGFsp, pH 1.2 (containing no drug), to 170 μL of each of the acceptor media to be used as a blind (no drug present) for the respective transfer wells. The setup of the plate is illustrated in Table II. Immediately after transfer, the plate was placed into a UV microplate reader (Spectramax® Plus^{384} used with SoftMax® Pro Software, version 4.8, Molecular Devices, Sunnyvale, CA, USA) which, besides reading samples, allowed temperature control and mixing. Experiments were run at 37±0.5°C, the 96-well plate was continuously shaken (interrupted for absorption measurements), and the amount

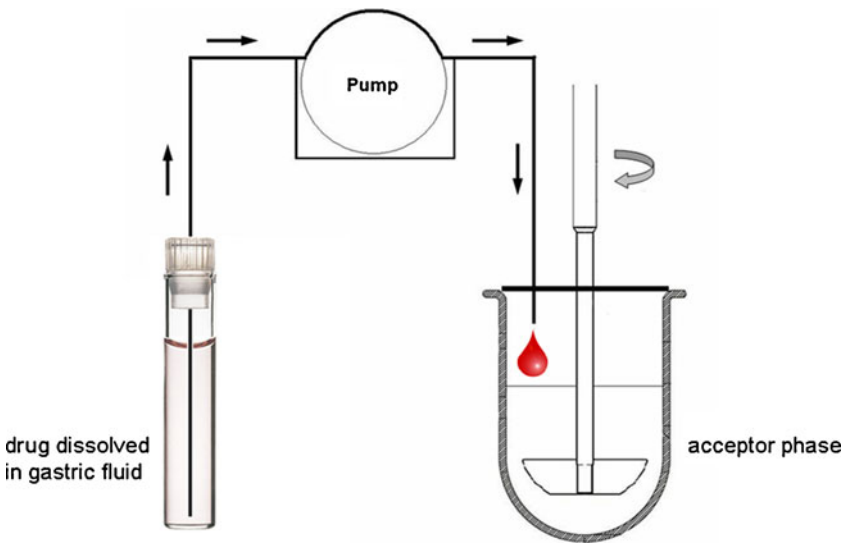

Fig. 2. Experimental setup for the mini-paddle transfer experiments

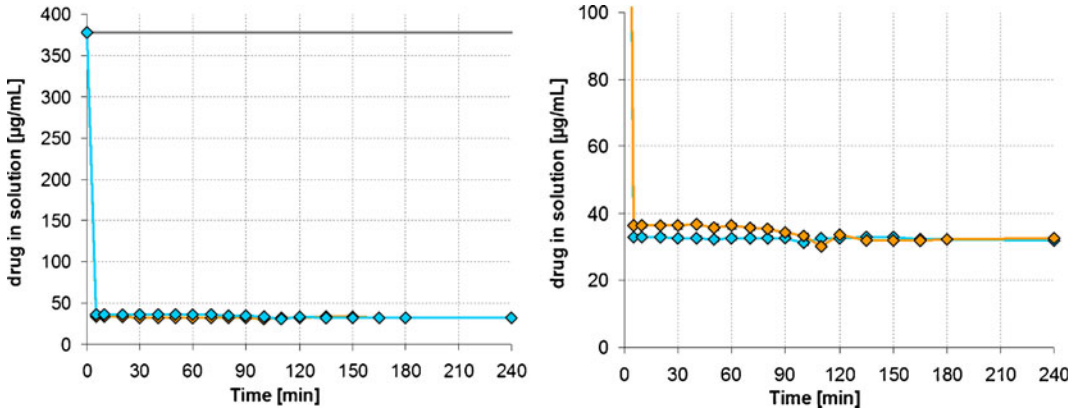

Fig. 3. Precipitation behavior (entire profile (*left panel*); detailed view (*right panel*)) of itraconazole in the 96-well plate transfer model in the simulated fasted (*orange*) and fed (*blue*) state, mean of 24±SD. The *gray line* indicates the theoretical drug concentration

of drug dissolved in each of the wells was monitored in 2-min intervals over a period of 4 h. As the use of the UV microplate reader provided the opportunity to scan a specific set of wavelengths for the entire 96-well plate in a matter of seconds, for each of the drugs, absorption was measured at two different wavelengths, namely, 256 and 400 nm for itraconazole and 275 and 400 nm for tamoxifen. The first wavelength was the maximum of absorption for the respective drug, and the wavelength of 400 nm was selected to account for any background absorption due to drug precipitation (background correction). The absorption (A) used for the quantification of the dissolved itraconazole and tamoxifen was corrected as follows: Corrected $A_{256\ nm}$ and $A_{275\ nm}$=($A_{256\ nm}$ or $A_{275\ nm}$ transfer well−$A_{256\ nm}$ or $A_{275\ nm}$ blind well)−($A_{400\ nm}$ transfer well−$A_{400\ nm}$ blind well).

Mini-Paddle Transfer Model

A miniaturized paddle transfer system (modified DT 600, Erweka, Heusenstamm, Germany) was used to simulate drug transfer from the stomach into the small intestine. For this objective, an appropriate amount of the formulation, containing 100 mg itraconazole or 10 mg tamoxifen (half of the standard dose), was completely dissolved in 40 mL SGFsp, pH 1.2 (donor phase). To simulate the worst case, i.e., a bolus of drug solution arriving in the upper small intestine, and also to generate a scenario comparable to that in the 96-well plate assays, in a first set of experiments, 40 mL of the donor phase was added to the acceptor phase, represented by 225 mL of either pH-modified FaSSIF, pH 7.5, or FeSSIF, pH 5.0, at the same time. In the second set of experiments, a peristaltic pump (Ismatec-type IPN, Glattbrugg, Zuerich, Switzerland) was used to continuously transfer the donor phase into the acceptor phase, which was agitated with a paddle speed of 100 rpm. A transfer rate of 2 mL/min (corresponding to 4 mL/min in the original setup) (6) was used to represent an average gastric emptying rate. All experiments were performed at 37°C. The experimental setup is illustrated in Fig. 2. The theoretical drug concentrations in the acceptor phase after complete transfer were the same as in the 96-well plate model, i.e., 377.5 µg/mL itraconazole and 37.75 µg/mL tamoxifen. Corresponding to the 96-well plate experiments, the amount of drug dissolved in the acceptor phase was monitored over a period of 4 h. For this purpose, the samples were taken at predetermined time points and, following appropriate dilution, assayed by HPLC. The HPLC system consisted of a LaChrom L-7100 pump, a LaChrom L-4250 UV–VIS detector, a LaChrom L-2000 autosampler, and EZChrom Elite Chromatography Data System software (Merck Hitachi, Darmstadt, Germany). Before analysis, all itraconazole samples were diluted by factor 5 using a

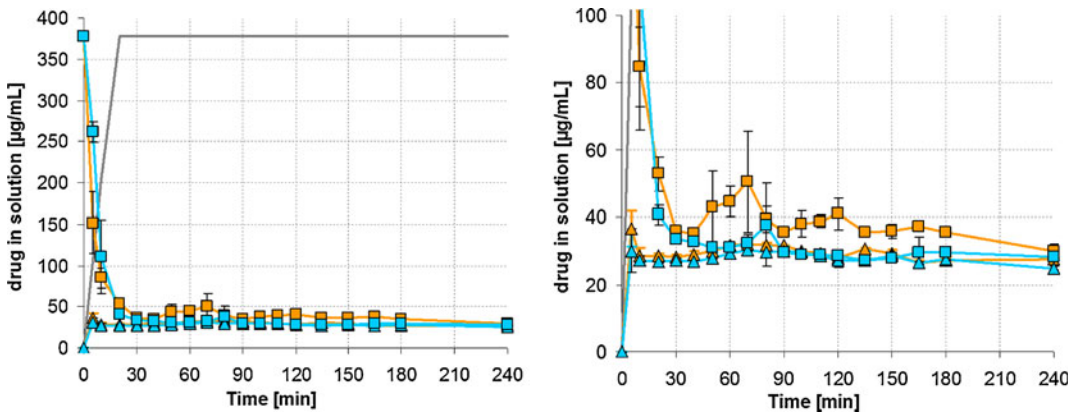

Fig. 4. Precipitation behavior (entire profile (*left panel*); detailed view (*right panel*)) of itraconazole in the mini-paddle transfer model after adding the gastric drug solution at the same time (*square*) or with a transfer rate of 2 mL/min (*triangle*) in the simulated fasted (*orange*) and fed (*blue*) state, mean of 3±SD. The *gray line* indicates the theoretical drug concentration

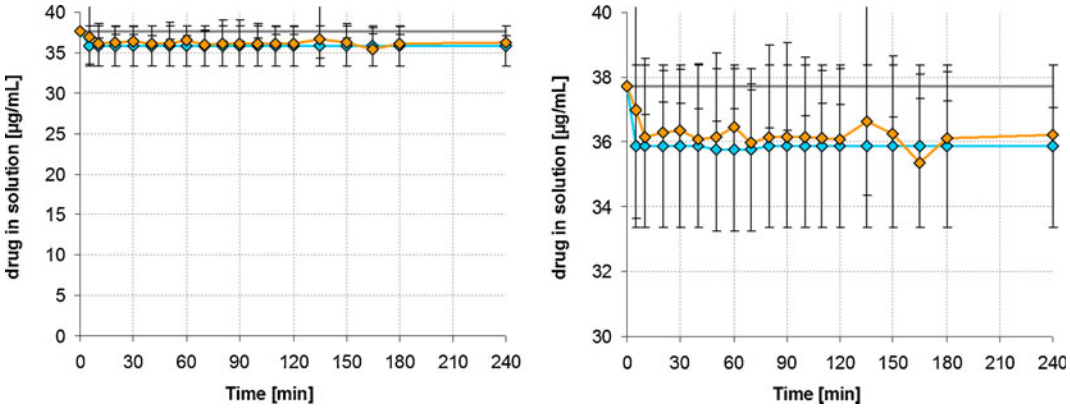

Fig. 5. Precipitation behavior (entire profile (*left panel*); detailed view (*right panel*)) of tamoxifen in the 96-well plate transfer model in the simulated fasted (*orange*) and fed (*blue*) state, mean of 24±SD. The *gray line* indicates the theoretical drug concentration

mixture of methanol/30 mM hydrochloric acid, 75:25. The analyses were performed on a Lichrocart® RP-18, 5-µm, 125×4-mm column (Merck, Darmstadt, Germany) using a mixture of acetonitrile/15 mM phosphate buffer, pH 3.0, 75:25 as the mobile phase. Flow rate was set at 1.2 mL/min, resulting in the elution of itraconazole at ~3 min. The amount of released drug was determined at a wavelength of 260 nm (9). The tamoxifen samples were diluted by factor 2 using also the methanol/30 mM hydrochloric acid 75:25 mixture. The analyses were performed on a Lichrocart® RP-18, 5-µm, 125×4-mm column (Merck) using a mixture of acetonitrile/15 mM orthophosphoric acid, pH 3.0, and triethylamine 80:19.9:0.1 as mobile phase. Flow rate was set at 1.0 mL/min, resulting in drug elution at ~2.7 min. The detection wavelength for tamoxifen was 275 nm.

RESULTS AND DISCUSSION

Results from both setups were very similar. Figures 3, 4, 5, and 6 represent the precipitation behavior of the two drug/HBenBCD complexes in the two different transfer systems simulating fasted and fed state dosing conditions.

The precipitation profiles given in Figs. 3, 4, 5, and 6 clearly indicate that the results from both setups were in very good agreement. Itraconazole precipitated quickly in both FaSSIF and FeSSIF (see Figs. 3 and 4). However, after complete precipitation, the amount of drug dissolved in the two media was in the same concentration range as in the 96-well plate experiments and overall independent on the method of donor phase addition. Moreover, the amount of drug dissolved in all media was significantly higher than the equilibrium solubility of the pure drug in these media. At this point, it needs to be emphasized that in all itraconazole solubility experiments, the equilibrium solubility of the drug itself was under the limit of quantification in all media with a pH of ≥1.8 (9). In the transfer experiments there was no significant difference in the concentrations of itraconazole dissolved in FaSSIF and FeSSIF after the test duration of 240 min. This observation is in good agreement with the results from the release experiments performed with the itraconazole/HBenBCD complex formulation, where the amount of drug dissolved in FaSSIF and FeSSIF was similar (9). No precipitation could be observed in the tamoxifen experiments, i.e., independent on the method of drug transfer; no precipitation of tamoxifen occurred over a time range of 4 h. This indicates that the drug is not likely to precipitate in the fasted and fed state small intestine when administered as an HBenBCD complex. As for itraconazole, these results are in good agreement with those from *in vitro* dissolution experiments (8).

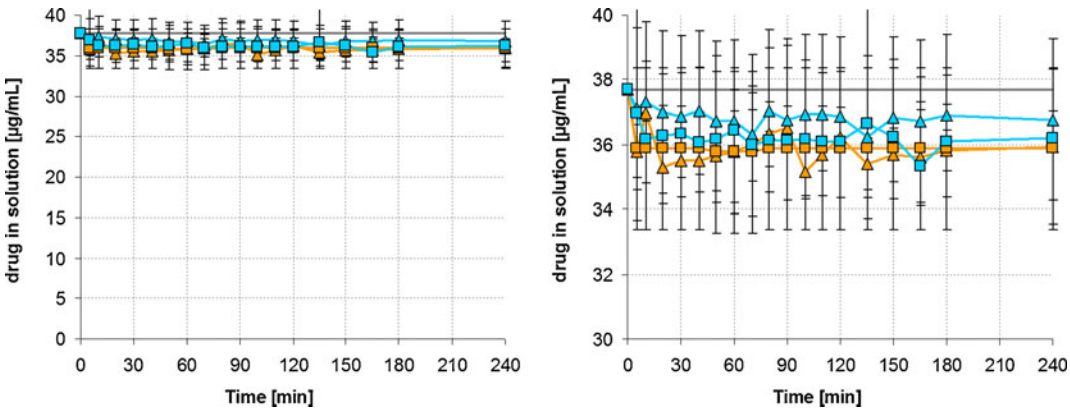

Fig. 6. Precipitation behavior (entire profile (*left panel*); detailed view (*right panel*)) of tamoxifen in the mini-paddle transfer model after adding the gastric drug solution at the same time (*square*) or with a transfer rate of 2 mL/min (*triangle*) in the simulated fasted (*orange*) and fed (*blue*) state, mean of 3±SD. The *gray line* indicates the theoretical drug concentration

In the present series of test, we used two formulations with extremely different solubility and dissolution and precipitation kinetics to see whether the two miniaturized models can be utilized to clearly distinguish between these formulations. The results obtained in our experiments were very promising, particularly for using these models in early formulation screening.

In our experiments, either a single average gastric emptying rate was used or the entire gastric drug solution was transferred into the small intestinal medium at the same time. However, it needs to be kept in mind that, particularly in the fasted state, gastric emptying, and therefore drug arrival into the intestine, can show significant variations depending on the motility pattern at the time of drug administration (6) and the precipitation kinetics can thus change as a function of the transfer rate. Therefore, if the intention is to screen for flow rate-derived changes in the precipitation kinetics, experiments would need to be performed using different transfer rates, which would hardly be possible with the 96-well plate model. Nevertheless, even if it might not be an adequate model for adequately predicting the precipitation rates, the 96-well plate transfer model represents an excellent fast and inexpensive tool in screening the extent of precipitation in early drug development.

CONCLUSION

Due to the possibility of generating physiologically relevant results but using smaller sample sizes and smaller volumes of media, both miniaturized transfer systems offer various advantages when evaluating the precipitation potential of poorly soluble weakly basic drug candidates. As in the very early stages of preformulation, the availability of new drug candidates is typically limited; particularly, the 96-well plate model represents a useful tool for this purpose.

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