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Predicting Food Effects on Drug Release from Extended-Release Oral Dosage Forms Containing a Narrow Therapeutic Index Drug

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INTRODUCTION

With the various types of oral extended-release (ER) dosage forms available, it is a challenge to accurately predict their in vivo behavior. If therapeutically appropriate, an oral ER dosage form should provide consistent drug release over the entire dosing interval, regardless of when it is given in relation to meal intake. Experience has shown, however, that this cannot be generally assumed to be the case. Substitution of one ER formulation for another or administering the same formulation under varying dosing conditions (e.g., fasted versus fed state) can have unexpected results. Unwanted effects that have been described during the last decades range from "dose-dumping" to sub-therapeutic plasma levels (*1–3*). As these unwanted side effects may result in severe risks for the patients, it would be highly desirable to be able to forecast the in vivo release rates under various dosing conditions using in vitro data.

The aim of these experiments was to develop an in vitro test method that can discriminate dissolution performance among ER dosage forms of a given drug, with view to predicting in vivo differences. Theophylline is a particularly suitable example for this purpose. This drug represents one of the cornerstones in the management of both the acute and chronic phases of reversible airway obstruction (*4*). However, because the drug has a narrow therapeutic index, the efficacy and toxicity of the active drug are highly dependent on plasma theophylline concentration (*5*). Optimum therapeutic serum level concentrations are generally considered to range from 8 to 15 µg/mL (*6–8*). The incidence of serious side-effects (ranging from nausea, vomiting, abdominal cramps, and diarrhea to arrhythmias, tremor, agitation, and convulsions) increases with concentration, particularly beyond the upper end of the usual therapeutic plasma theophylline range (*5*, *8*). Toxic symptoms commonly occur at serum concentrations greater than 25 µg/mL but are typically not noted below 15 µg/mL (*8*). Thus, it is very important to maintain serum drug levels in the therapeutic range.

When given as a solution or an immediate-release (IR) dosage form, theophylline is rapidly and completely absorbed along the small intestine and the colon (*9–12*). As the elimination half-life of theophylline is short (4–9 h), 4- to 6-hourly administration of IR dosage forms is required to maintain the serum concentration within the therapeutic range (*12*). With such a regimen, lack of patient compliance is a serious problem, and low trough levels in the morning, with a possible risk of breakthrough of symptoms, can occur (*13*). Accordingly, ER dosage forms are the formulations most favored for the long-term management of chronic bronchospasm. Given only once or twice daily, they should result in more constant plasma levels, increase patient compliance, and avoid therapeutic gaps while preventing serious side effects.

An ideal ER product should demonstrate complete bioavailability, minimal fluctuations in drug concentration at steady state, reproducibility of release characteristics independent of food, and minimal diurnal variation. However, with the first ER formulations, it became clear that not all meet the requirements of an ideal theophylline ER product. It was shown that in many cases, drug release from various theophylline ER formulations could be influenced (either increased or decreased) by concomitant intake of food (*1*, *3*, *14–18*). Although in maintenance therapy of *chronic* obstructive lung disease, most drugs are given in conjunction with food, the recent literature contains very few in vivo studies (*1*, *5*, *13*, *18–20*) and next to no in vitro investigations (*21*) of the influence of food on the bioavailability of theophylline from ER formulations. Food intake can influence the rate of drug release from the dosage form, the rate of drug absorption, the amount of drug absorbed, or all of these parameters simultaneously. The rate of drug release of various ER formulations can be affected by the composition of the intraluminal contents, which itself is at least partly determined by the size and the composition of the co-administered meal. Depending on the type of dosage form and the intraluminal conditions, a co-administered meal can result in both so-called "positive" and "negative" food effects. Positive food effects typically come along with an increase in drug release from ER formulations and in the worst case, can represent a great risk for the patient, particularly when a large amount of the dose is dumped within a short period of time (*22*, *23*). Such positive food effects are often the result of the loss of the integrity of matrices or coatings (i.e., devices that control drug release of ER dosage forms). It has been observed that fats, high concentrations of bile components, and pH changes (*18*, *22*) are typical triggers for increased drug-release rates.

However, the same factors can also result in the opposite, a so-called negative food effect, which comes along with a decrease in drug release. In addition, there are many other reasons for negative food effects, such as adsorption to food contents, decreased luminal diffusivity due to an increase in viscosity in the upper GI tract, and changes in the absorption rate due to food-induced changes in GI-motility and passage time along the GI tract (*16*, *24*). The latter changes will most likely not have an impact on the absorption process itself but can result in a significant change in the maximal plasma concentration (C_{max}) and the time to maximal plasma concentration (t_{max}) (*19*). Overall, food-induced changes in the intraluminal milieu can result in an unexpected shift in plasma theophylline concentration. Comparing once-daily and twice-daily preparations, pronounced food effects may have an even greater impact with once-daily preparations, because the total daily dose is ingested in a single administration. Thus, sudden release of the entire ER dose (dose-dumping) can and does result in toxic plasma concentrations (*1*), whereas a decrease in drug-release rate and the total amount released can come along with subtherapeutic plasma levels.

The main objective of this series of tests was to examine whether it is possible to detect the influence of food on drug release of different ER theophylline formulations using in vitro dissolution methods. The USP describes various dissolution methods for examining drug release from theophylline ER products (*25*). However, the test conditions and specifications are extremely heterogeneous. Attempts to use compendial dissolution methods for predicting theophylline absorption from sustained-release dosage forms have been made (*16*, *19*, *26*, *27*). In most cases, the paddle or basket apparatus and simple aqueous buffers like 0.1 N hydrochloric acid or phosphate buffer have been used, and the researchers only took into account typical pH values in the fasted stomach and small intestine. However, since these methods are not capable of simulating the critical physiological conditions with respect to pH values, passage times through different sections of the gastrointestinal (GI) tract, or the presence of food or bile components, they often failed (*15*, *16*); the resulting profiles did not show a close relationship to either rate or extent of absorption.

For these reasons, the BioDis methodology (*28–30*), combined with a series of media reflecting fasted- and fed-state environments along the GI lumen (*31*), was used to predict drug release from theophylline formulations currently available on the European market, under different dosing conditions.

MATERIALS AND METHODS

Materials

Theophylline drug substance (lot # 102K0547) was purchased from Sigma-Aldrich, Steinheim, Germany. All tested dosage forms were kindly donated by their German manufacturers (Aliud Pharma GmbH & Co KG; Laichingen; 3M Medica, Neuss; Lindopharm, Hilden; Trommsdorff GmbH & Co, Alsdorf, Germany). Taurocholic acid sodium salt (PCA code 2012, lot # 20000060181) was purchased from Tiefenbacher (Hamburg, Germany). Egg lecithin (Lipoid E PC, lot# 105026-1) was kindly donated from Lipoid GmbH (Ludwigshafen, Germany). All other chemicals were of analytical grade or equivalent, and purchased commercially.

Various ER formulations, each containing 300 mg of theophylline, were chosen at random and tested. The results for two tablet formulations and one capsule (pellet) formulation will be discussed. For the encapsulated pellet product, in both paddle and BioDis experiments, the pellets were removed from the capsules before testing to prevent clogging of the mesh screens of the BioDis apparatus. The formulations and their compositions are given in Table 1.

Experimental Conditions

The test setups used in the present study were designed to simulate the fasted and fed conditions of the GI tract. Preliminary experiments were performed with the paddle setup to check if it would be possible to generate predictive dissolution profiles with a standard dissolution setup (i.e., the paddle and a single dissolution medium reflecting pH conditions of the mid jejunum). All experiments were run at least in triplicate, and results are expressed as percentage mean $\pm$ SD dissolved at each sampling time.

Paddle Experiments

In a preliminary set of experiments, dissolution tests were performed with the paddle apparatus using conditions similar to those described in the USP: a stirring speed of 50 rpm, 900 mL of simulated intestinal fluid (SIFsp USP 30) with a pH of 6.8, and a test duration of 8 h.

Table 1. Composition of the Formulations Studied

Product	Excipients
Pellet composition	
Theophyllin AL 300 retard, Aliud Pharma, Batch # 21915	dibutylphthalate, corn starch, poly(O-ethyl)-cellulose, povidone, saccharose, shellac, talcum, titanium dioxide (E171), iron oxide (E172)
Tablet composition	
Contiphyllin 300 mg, Lindopharm, Batch # 130102	poly(O-2-hydroxypropyl, o-methyl)cellulose, shellac, calcium hydrogenphosphate, silicium dioxide, magnesium stearate
Tromphyllin retard 300 mg, Trommsdorff, Batch # 209528	hypromellose, polyethylenglycol 6000, magnesium stearate

Table 2. Dissolution Media and Transit Times Used in Fasted Gradients

	Media and pH				Residence time (min)	
GI-segment	**compendial media**	**pH**	**biorelevant media**	**pH**	**tablets**	**pellets**
Stomach	SGFsp[a]	1.8	FaSSGF[a]	1.8	60	30
Proximal Jejunum	Blank FaSSIF	6.5	FaSSIF	6.5	15	45
Distal Jejunum	Blank FaSSIF[a]	6.8	FaSSIF[a]	6.8	15	45
Proximal Ileum	Blank FaSSIF[a]	7.2	FaSSIF[a,b]	7.2	30	45
Distal Ileum	Blank FaSSIF[a]	7.5	Blank FaSSIF[a]	7.5	120	45
Proximal Colon	SCoF	5.8	SCoF	5.8	240	240

[a] pH modified, [b] concentration of bile components modified

Samples were removed at predetermined time points, and following filtration through a 0.45-µm Teflon filter, they were analyzed by HPLC.

BioDis Experiments

Further experiments were performed with the BioDis apparatus. The vessels were filled with 200 mL of media at 37 ± 0.5 °C. Mesh sizes of 420 µm were used for both the top and bottom mesh.

Fasted-State Gradients

In the first set of experiments, the passage through the upper GI tract and proximal colon was simulated using a compendial pH-gradient method (*28*, *29*) to check whether varying pH conditions influences drug release in the GI tract. Next, a corresponding test was performed using biorelevant media to simulate additional parameters that may affect drug release during GI passage. In contrast to fasted-state gradients that have been used before (*28*, *32*, *33*), a gradient of Blank Fasted-State Simulated Intestinal Fluid (FaSSIF) media (i.e., FaSSIF media without bile components) (*34*) was used to simulate the pH changes during small intestinal passage. Apart from the surface tension, the Blank FaSSIF media have exactly the same physicochemical properties as the corresponding FaSSIF media. Therefore, differences in the dissolution behavior in FaSSIF and Blank FaSSIF clearly display the impact of bile components on drug release. Whereas in previous studies, SGF_{plus} was used as a "biorelevant" medium to simulate gastric conditions in the fasted state, in this study, it was replaced by Fasted-State Simulated Gastric Fluid (FaSSGF) (*35*), a new medium that, compared with previously proposed media, constitutes a more accurate simulation of fasting gastric contents. Another medium utilized was Simulated Colonic Fluid (SCoF) (*36*), which has a composition and physicochemical characteristics that come closer to in vivo conditions than the media used before.

Mean transit times reported in several gamma-scintigraphy studies (*37*–*42*) were utilized to simulate fasted-state residence times in the different regions of the GI tract. Further, the different gastric residence times of tablets and small particles (≤ 2 mm) were accounted for in the dissolution model (*37*, *40*, *42*). Additionally, to simulate the reuptake of bile acids along the lumen of the small intestine (SI), it was necessary to develop a gradient of bile component concentrations to simulate SI passage adequately. A dip rate of 10 dpm was used to simulate fasted-state motility. The test duration was 8 h in all experiments. Table 2 illustrates the test conditions that were used to simulate gastrointestinal passage with compendial and biorelevant media.

Table 3 shows the pH values and the corresponding concentrations of sodium taurocholate and lecithin that were used to create a biorelevant pH gradient.

Fed-State Gradients

To achieve the main objective of the studies (i.e., to check whether drug release from the different dosage forms is influenced by fasted- versus fed-state dosing conditions), two new gradient methods were designed to simulate passage through the fed-state GI tract after a standardized high-fat breakfast. Similar to fasted-state experiments, a gradient of Blank Fed-State Simulated Intestinal Fluid (FeSSIF) media (i.e., FeSSIF media without

Table 3. pH Values and Bile Salt Concentrations Used to Simulate Passage Through the Fasted Small Intestine

GI segment	pH	Biorelevant media	Sodium taurocholate	Lecithin
Proximal Jejunum	6.5	FaSSIF	3 mmol/L	0.75 mmol/L
Distal Jejunum	6.8	FaSSIF[a,b]	3 mmol/L	0.75 mmol/L
Proximal Ileum	7.2	FaSSIF[a,b]	1.5 mmol/L	0.375 mmol/L
Distal Ileum	7.5	Blank FaSSIF[a]	no	no

[a] pH modified [b] concentration of bile components modified

bile components) was used to simulate to simulate the pH changes during small intestinal passage (*34*). Thus, differences in the dissolution behavior in FeSSIF and Blank FeSSIF also clearly display the impact of bile components on drug release. Mean transit times reported in several gamma-scintigraphy studies were utilized to simulate fed-state residence times in the different regions of the GI tract (*37*, *39*, *43*, *44*), and again, the different gastric residence times of tablets and small particles were accounted for in the dissolution model. The gastric residence time is typically much longer for single-unit than for multiple-unit dosage forms, but the residence times in the different sections of the small intestine are not influenced that much by the size of the dosage form. Analogous to the previous studies, the passage through the upper GI tract was first simulated using a compendial pH gradient, and then a corresponding test was performed using biorelevant media to simulate additional parameters that may be crucial for in vivo drug release. In particular, the osmolality, buffer capacity, and rate of bile secretion are subject to food-induced changes, and it was considered important to adequately simulate these food-related changes in vitro. Additionally, as in the fasted-state experiments, the reuptake of bile acids in the small intestine was simulated by decreasing the bile salt concentration along the simulated SI passage. The test duration was again 8 h for all experiments. To represent the increased motility that would be expected in the postprandial state, the dip rate was increased slightly to 15 dpm for all sections of the GI tract. The samples were removed periodically using a plastic syringe and immediately filtered through a 0.45-µm Teflon filter.

Table 4 shows the pH values and the corresponding media that were used to generate the compendial and the biorelevant gradients.

Table 5 shows the pH values and the corresponding concentrations of sodium taurocholate and lecithin that were used to generate the biorelevant gradient for the fed state.

Table 5. pH Values and Bile Salt Concentrations Used to Simulate Passage Through the Fed Small Intestine

GI-segment	pH	Biorelevant media	Sodium taurocholate	Lecithin
Proximal Jejunum	5.0	FeSSIF	15 mmol/L	3.75 mmol/L
Distal Jejunum	6.5	FeSSIF[a,c]	15 mmol/L	3.75 mmol/L
Proximal Ileum	6.5	FeSSIF[a,b,c]	7.5 mmol/L	1.875 mmol/L
Distal Ileum	7.5	Blank FaSSIF[a]	no	no

[a] pH modified, [b] concentration of bile components modified, [c] phosphate buffer

HPLC Analysis

All samples were analyzed by HPLC. Because various media of different compositions and pH were used to generate both the fasted- and the fed-state gradient, it was necessary to perform some sample preparation before injection into the HPLC. In so doing, it was intended to establish an efficient and cost-effective, yet selective and reproducible method that would be applicable to all samples in biorelevant media. Two parameters appeared to be crucial to obtain equal conditions for HPLC analysis: the pH and buffer capacity of the mobile phase and the sample itself. For the purpose of this study, the pH and buffer capacity of the sample were modified. An acetate buffer with a pH of 5.0 seemed to be suitable to adjust the pH of each sample before analysis. The pK_a values of theophylline are $pK_{a1} = 0.3$ and $pK_{a2} = 8.6$ (*45*), so a pH in the range of 4–5 seemed ideal to keep theophylline in solution. Therefore, Blank FeSSIF was chosen to dilute all samples, because it has a pH of 5.0 and, moreover, a high buffer capacity, which guarantees the resulting sample pH to be robust during analysis. As Blank FeSSIF has to be prepared in the course of making the biorelevant dissolution media anyway; its use for sample dilution

Table 4. Dissolution Media and Transit Times Used in the "Fed-State" Theophylline Study

	Media and pH				Residence time (min)	
GI-segment	compendial media / buffers	pH	biorelevant media	pH	tablets	pellets
Stomach	A) Acetate buffer / SGFsp[a]	5.0/2.0	B) Ensure Plus or Milk	6.5	A) 120/120; B) 240*	120
Proximal Jejunum	Blank FeSSIF	5.0	FeSSIF	5.0	15	45
Distal Jejunum	Blank FeSSIF[a,c]	6.5	FeSSIF[a,c]	6.5	15	45
Proximal Ileum	Blank FeSSIF[a,c]	6.5	FeSSIF[a,b,c]	6.5	30	45
Distal Ileum	Blank FeSSIF[a,c]	7.5	Blank FaSSIF[a]	7.5	120	45
Proximal Colon	SCoF	5.8	SCoF	5.8	–	240

[a] pH modified, [b] concentration of bile components modified, [c] phosphate buffer
* to better screen gastric performance of formulations, samples should be taken at least every 60 min

saved both time and costs.[5] Thus, before analysis, all samples were diluted 1:10 with Blank FeSSIF.

Because of the complex composition of Ensure Plus, samples from this medium required an additional preparation step before dilution and analysis (i.e., the aqueous phase had to be separated from the lipid phase and the proteins). Because it was difficult to predict whether theophylline would concentrate in the aqueous, lipid, or pellet phase or distribute in all of them, an adequate amount of theophylline standard substance was treated in the same manner as the samples and used as the reference for calculating the amount of drug released in the stomach. Samples and standard were processed as follows:

- 300.0 mg of theophylline standard substance (nominal drug load of one tablet) was added to 200.0 mL of Ensure Plus and stirred for 240 min ($n = 6$). The resulting mixture represented the "100% reference."
- Samples and reference were transferred to 1.5 mL caps (6 caps per sample) and centrifuged for 30 min at a rotation speed of 20,500 rpm to result in phase separation (see Figure 1).
- The aqueous phases ($n = 6$ per sample) were pooled and filtered twice, first with a 5-µm polyamide (PA) filter and then with a 0.45-µm Teflon (PTFE) filter.
- 100 µL of the resulting filtrate was diluted 1:10 with Blank FeSSIF and analyzed by HPLC.

The analyses were performed on a Lichrocart RP-18, 5-µm, 125 × 4 mm column (Merck, Darmstadt, Germany), using a mixture of 20:80 methanol/purified water as mobile phase. Because the pH of all samples was adjusted with Blank FeSSIF, it was possible to use this simple methanol/water mixture as the mobile phase, which also proved to be cost-effective. The flow rate was set at 1.2 mL/min, resulting in elution of theophylline at ≈ 4 min in all cases. The amount of released drug was determined at 254 nm.

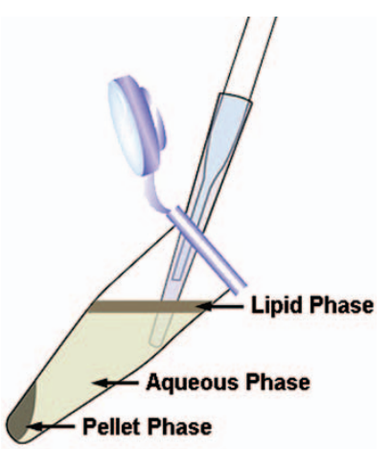

Figure 1. Phase separation after centrifugation of Ensure Plus samples.

The HPLC method had been validated before use. For all media, the method was linear over a concentration range of 3.5–180 µg/mL ($r^2 \geq 0.999$). The limits of detection and quantification were 1.5 µg/mL and 2.5 µg/mL, respectively. The recovery of the method was between 98% and 102.5%. A standard curve was used to calculate drug release in compendial and bile-salt containing media, whereas the total amount of drug released in milk and Ensure Plus was calculated as follows:

$$FS[\%] = \frac{PA_S}{PA_R} = *100[\%]$$

where FS[%] is the fraction of dose released under simulated fed-state gastric conditions, PA_S is the peak area resulting from direct sampling in Ensure Plus, and PA_R is the peak area resulting from the reference (i.e., 300.0 mg of theophylline standard substance dissolved in 200 mL of Ensure Plus) (mean of $n = 6$).

In Vitro Dissolution Profile Comparison

Where applicable, the similarity factor f_2 (*46*, *47*) was calculated to indicate similarity of dissolution profiles under different test conditions. The f_2 value was calculated as follows:

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where log is logarithm base 10, n is the number of sampling points, Σ is the summation over all time points, and R_t and T_t are the cumulative percentage dissolved at each of the selected time points of the reference and test product, respectively. When the two profiles are identical, $f_2 = 100$. An f_2 value greater than 50 was the criterion for similarity between two dissolution profiles.

RESULTS AND DISCUSSION

Paddle Experiments–Compendial Media

Although all of the tested theophylline ER dosage forms are approved in Germany for the same indication, even under "quality control conditions" in the paddle system, some dosage forms exhibited different release patterns (see Figure 2).

To help clarify the issue of robustness of drug release rate in the GI tract, it was also necessary to perform additional experiments that more closely simulate the conditions in the human GI tract.

BioDis Experiments–Compendial Media

The same medium (SIFsp USP 30, pH 6.8) but a different apparatus, the BioDis, was used to check whether a different hydrodynamic pattern might influence drug release from the various theophylline ER formulations. With a view to performing a series of tests using the pH-gradient method so that results in SIPsp pH 6.8 could

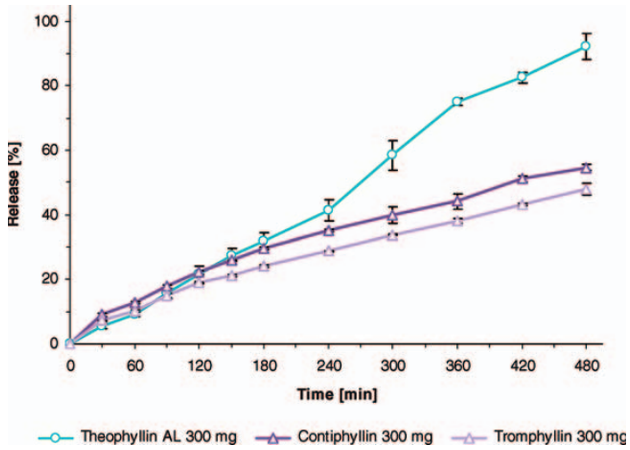

Figure 2. Dissolution behavior of different theophylline ER dosage forms created with the paddle apparatus in SIFsp pH 6.8.

be compared with those from the pH-gradient experiments, residence times in different vessels were adapted to mean physiological passage times in the fasted state. Despite these changes, the resulting dissolution profiles were mostly similar to those with the paddle apparatus (see Figure 3). However, in contrast to the paddle results, the drug release rate from Contiphyllin was slightly increased in the BioDis tests.

Unfortunately, since the BioDis sampling times were chosen to correspond to those in the subsequently performed pH-gradient experiments, there was an insufficient number of sampling times that corresponded to those from the paddle experiments. Thus, it was not possible to calculate the f_2 factor for dissolution profile comparison. However, the results suggest that, in contrast to the pellet formulation, drug release from the tablet

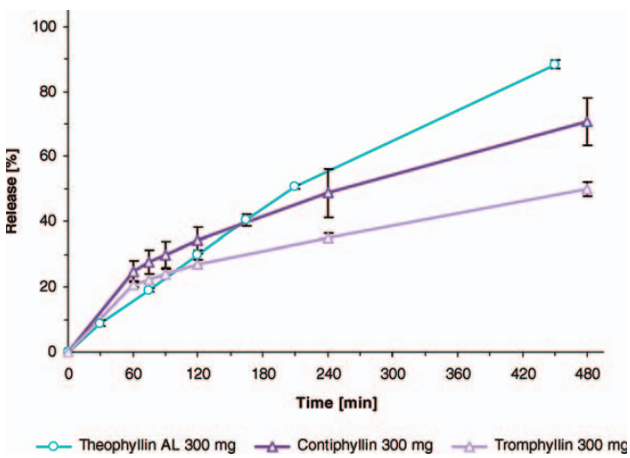

Figure 3. Dissolution behavior of different theophylline ER dosage forms created with the BioDis apparatus in SIFsp pH 6.8.

formulations is more dependent on the test conditions and therefore might be more sensitive to different dosing conditions (i.e., fasted- vs fed-state dosing). To predict drug release after ingestion of the dosage form under different dosing conditions, a single medium would not be sufficient. Hence, in the subsequent series of experiments, drug release was examined using compendial and biorelevant pH gradients to simulate pH conditions in the fasted- and fed-state stomach and to check whether the changing composition of the intestinal contents might influence drug release of the dosage forms studied.

BioDis Experiments–Compendial and Biorelevant Media Fasted versus Fed State

In the final set of experiments, compendial and biorelevant pH gradients were used to simulate the pH and composition of the GI contents under fasted- and fed-state conditions. The resulting profiles indicate that the theophylline ER formulations vary in their sensitivity to different dosing conditions. From a comparison of release profiles generated with the fasted- and fed-state compendial gradient, it was obvious that none of the dosage forms exhibited pH-dependent drug release in the gastrointestinal pH range. The resulting profiles from the two compendial pH gradients were nearly superimposable for all dosage forms tested. Similar to the experiments in single media, an f_2 calculation could not be used to directly compare profiles generated with the fasted-state gradients with those from the fed-state gradients since the number of samples taken at corresponding sampling times was not sufficient (< 3). However, based on the release profiles shown in Figures 4–6, one could estimate that for each of the dosage forms tested, f_2 would be greater than 50, not close to 100 when comparing drug release in the fasted- and fed-state compendial gradients.

Using biorelevant pH gradients to mimic not only pH but also further aspects of the composition of the different GI fluids, the release patterns in the (simulated) fasted and fed state were dependent on the composition of the media and the corresponding residence times (see Figures 4–6).

Figures 4 illustrates that drug release from Theophyllin AL was not affected by varying the pH conditions to represent fasted- and fed-state conditions in the human GI tract. Drug release in the compendial pH gradients resulted in superimposable release profiles that exhibited nearly zero-order kinetics. This was not surprising as the pellet formulation is coated with ethylcellulose (EC), a polymer with pH-independent release properties.

Drug release was only slightly increased using a biorelevant pH gradient to simulate fasted-state conditions in the GI tract, whereas in biorelevant media representing the fed-state, release was slightly slower. Again, this might result from the use of EC as coating agent of the pellets. Based on these observations, it is

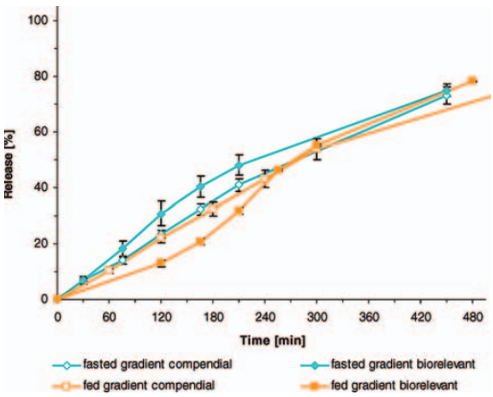

Figure 4. Dissolution profiles of Theophyllin AL 300 retard pellets under fasted- and fed-state conditions.

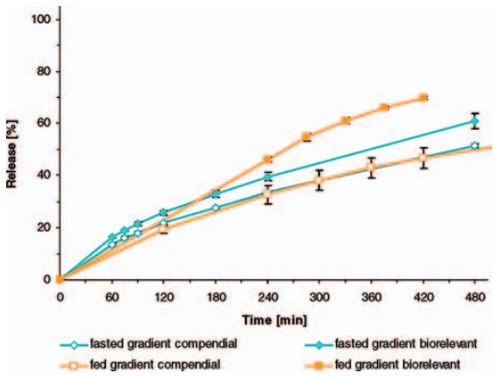

Figure 5. Dissolution profiles of Contiphyllin 300-mg tablets under fasted- and fed-state conditions.

obvious that buffer capacity, ionic strength, and content of lipophilic compounds in the dissolution medium have only a minor impact on the swelling of the EC film. Results further indicate that even in the presence of high viscosity and fat (i.e., in Ensure Plus), there is little change in release characteristics. The slight decrease in observed drug release corresponds to both in vivo observations made with similar dosage forms (i.e., coated theophylline pellets, where the absorption rate was reduced after coadministration with a high-fat breakfast, most likely due to a decreased release rate), and results from an in vivo bioavailability study (*16*, *48*, *49*). In this bioavailability study, the formulation was administered either in the fasted state or together with a high-fat breakfast. Following administration, the AUC, C_{max}, and t_{max} were evaluated. Results indicate that the AUC was not significantly affected by food intake (AUC fasted 162.24 ± 57.68 µg/mL·h vs AUC fed 168.12 ± 55.00 µg/mL·h), whereas a slight shift in C_{max} (C_{max} fasted 6.81 ± 1.86 µg/mL vs C_{max} fed 7.91 ± 1.74 µg/mL) and t_{max} (t_{max} fasted 7.56 ± 1.42 h vs t_{max} fed 9.28 ± 1.74 h) became obvious. These results indicate that concomitant food intake does not significantly alter theophylline bioavailability from Theophyllin AL but might have a slight impact on the release rate, which in turn determines the in vivo absorption rate of the drug. Overall, these observations correlate well with the results obtained in our in vitro study.

As for the pellet formulations studied, drug release from the tablet formulations was independent of pH. However, in contrast to the pellet dosage forms studied, both tablet formulations showed higher release rates in the biorelevant media that simulate fed-state conditions (see Figures 5 and 6).

Concentrations of bile components corresponding to those of the fasted intestinal lumen led to merely a slight increase in drug release from both tablet formulations. Increasing the concentration of bile components to those typical of the fed state, drug release further increased.

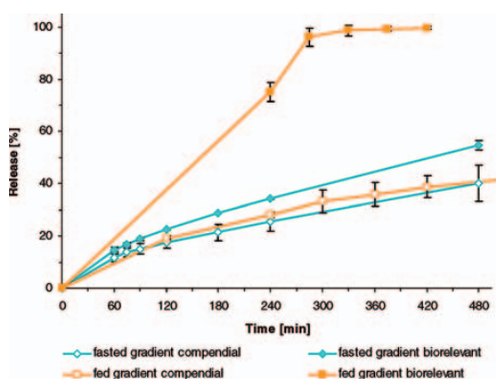

Figure 6. Dissolution profiles of Tromphyllin retard 300-mg tablets under fasted- and fed-state conditions.

For Contiphyllin, the increase was relatively modest. For Tromphyllin tablets, however, results generated with the biorelevant gradients indicate that dose dumping may occur when the tablet is taken with or after a meal (i.e., the FDA high-fat standard breakfast). This would be associated with a pronounced increase in the rate of absorption, placing the patient at a greater risk of toxicity. The f_2 values calculated to compare results from compendial and biorelevant gradients are in good agreement with these observations (see Table 6).

Whereas the presence of bile components or food seemed to have only a modest impact on drug release from the Theophyllin AL pellets, the tablet formulations proved to be more sensitive to changes in media composition. Particularly under fed-state conditions, the drug release rate from the tablets increased and the release profiles were different ($f_2 < 50$) from those obtained in the compendial media. For the Tromphyllin product, this food effect was so pronounced that it was not even possible to calculate f_2, since in contrast to experiments in compendial

Table 6. Comparison of Dissolution Profiles in Compendial and Biorelevant Media

	Theophyllin AL 300 retard	Contiphyllin 300 mg	Tromphyllin retard 300 mg
Fasted compendial vs biorelevant	62.32	63.29	56.13
fed compendial vs biorelevant	53.09	37.53	*

* f_2 could not be calculated since as early as after 120 min, more than double the amount of drug was released (compendial: 28.09%, biorelevant: 77.76%) in biorelevant media compared with compendial media

media where the average amount released was approximately 28% within the first two hours, in biorelevant media (i.e., Ensure Plus), about 78% of the dose was released within the same period of time.

Based on the package insert information about the composition, the two formulations had been expected to behave similarly. Therefore, a second set of experiments was performed to verify the drug-release rate in the postprandial stomach. The main objective of this series of tests was to check whether the release of almost 80% of the active drug during gastric residence occurred via dose dumping or if drug release occurred at a steady state over the course of gastric residence. Drug-release profiles of Contiphyllin and Tromphyllin tablets were generated under postprandial gastric conditions (i.e., Ensure Plus) for 240 min, where sampling took place every 60 min, are summarized in Figure 7.

Dissolution profiles clearly indicate that food effects on drug release from Tromphyllin did not result in a bolus dose dumping but rather in a much higher but still zero-order drug-release rate.

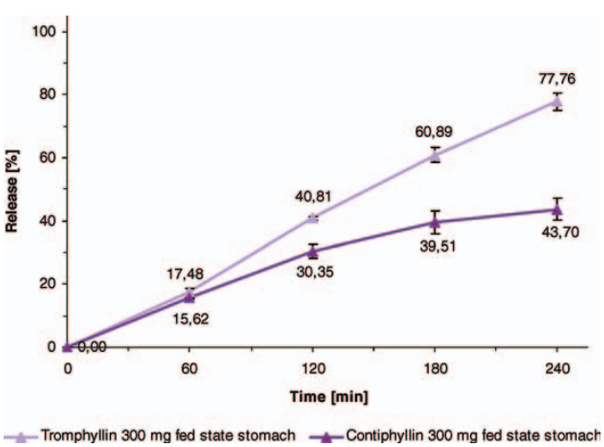

Figure 7. Drug release rates of Contiphyllin 300 mg and Tromphyllin retard 300-mg tablets under fed-state gastric conditions.

Package insert information (*50*) indicates that Contiphyllin tablets consist of a matrix with theophylline embedded in hydroxypropylmethylcellulose (HPMC), a highly swellable, hydrophilic polymer that represents the release controlling device. When in contact with GI fluids, the HPMC matrix becomes hydrated and starts to gel.

Based on the information from the manufacturer (*51*), the release principle used in Tromphyllin retard 300-mg tablets is similar to that described for Contiphyllin 300-mg tablets. Theophylline is embedded in a patented Hydrotard matrix made of HPMC with polyethylene glycol (PEG 6000) added as a pore-forming agent. As described for Contiphyllin, the matrix starts to gel when it comes in contact with GI fluids. Theophylline is then released via both diffusion and gradual erosion of the matrix itself. Nevertheless, the two apparently similar tablet formulations behaved differently under conditions simulating passage through the fed-state GI tract.

Closer examination revealed that although the matrices that comprise Contiphyllin and Tromphyllin tablets consist of the same class of polymer (HPMC) embedding the same dose (300 mg) of theophylline, the excipients differ in both type and amount. The tablets differ also in dimension and weight. Further, Tromphyllin contains PEG 6000 while Contiphyllin does not.

The impact of variable amounts of highly water-soluble fillers (e.g., PEG 6000) on in vitro and in vivo drug release from HPMC matrix systems has been examined in various studies (*52*, *53*). Results of these studies indicate that drug release from such matrices is strongly depending on both the viscosity of the matrix-forming agent (HPMC) and the amount of hydrophilic filler that is dispersed in the matrix. Typically, a purely diffusion-controlled release can be observed from matrices of highly viscous HPMC types containing low concentrations of PEG 6000. In contrast, matrices made of lower viscous types or containing higher amounts of hydrophilic fillers are more susceptible to disintegration and, thus drug release from such formulations is often controlled by both diffusion and erosion.

A diffusion- and erosion-controlled drug release can also be a result of the weak integrity of the gel layer of the tablet that might derive from high drug load of the matrix. Such effects have, for instance, been reported from Colombo et al. (*54*, *55*) who studied the gel-layer thickness in HPMC matrices loaded with increasing amounts of a soluble drug. In these studies, matrices with a very high drug load ($\geq$80%) were more sensitive to erosion and water penetration than matrices containing small amounts of drugs. Colombo et al. considered that drug release from highly loaded, swellable HPMC matrices was controlled by swelling, diffusion, and erosion fronts in the hydrated matrix.

Based on all observations mentioned above, the matrix of Tromphyllin, which contains about 82% theophylline and an unknown amount of a hydrophilic filler, is even

more likely to be sensitive to erosion because the active drug is theophylline, which recently has been shown to reduce the entanglement of polymeric chains and to lower gel resistance when present in high concentrations (*56*).

A further parameter to be considered in terms of predicting drug release from HPMC is ionic strength. Since increasing electrolyte concentration can prevent gel formation of various matrices (*57*), it was hypothesized that the higher concentrations of various electrolytes in the Ensure Plus medium may have contributed to loss of the integrity of the gel layer of Tromphyllin, resulting in faster erosion of the matrix. This hypothesis was supported by the results of an additional test in which both tablet formulations ($n = 3$) were moved in Ensure Plus or Blank FeSSIF–SGF pH 2 for 4 h (2 h/2 h) under conditions corresponding to those in the drug-release experiments. After 4 h, the tablets were taken out of the glass cylinder and inspected. As expected for a diffusion-controlled drug release independent of the test medium, Contiphyllin tablets, which contain a drug/polymer ratio of approximately 1:1, were swollen but still intact (see Figure 8). Using compendial media, the same was observed for Tromphyllin tablets (see Figure 8). By contrast, approximately half of the original matrix was lost by erosion within the same time frame from Tromphyllin tablets in Ensure Plus, with correspondingly high drug release (see Figures 7 and 8). Even the use of smaller dip rates (i.e., 5 dpm and 10 dpm) in the gastric medium (data not shown here) resulted in similar release profiles. This performance clearly indicates that the in vivo drug release from Tromphyllin might be sensitive to different dosing conditions and that higher theophylline blood levels might be measured after administration with a high-fat meal.

Figure 8. Contiphyllin 300-mg (left-hand side) and Tromphyllin retard 300-mg tablets (right-hand side) before and after a test duration of 4 h in compendial media (bottom) and Ensure Plus (top).

In 1997 a steady-state study comparing fasted-state bioavailability of Tromphyllin retard 300 mg and a reference product (brand name not published) was performed in 18 subjects (*51*). AUC, $C_{max/ss}$, $C_{min/ss}$, and peak-trough fluctuation (PTF) after Tromphyllin administration were comparable with those of the reference product. The study was performed to examine fed-state pharmacokinetics, but the only parameter that was published was $C_{max/ss}$. For Tromphyllin, fed-state $C_{max/ss}$ was compared with that resulting from fasted-state administration ($C_{max/ss}$ fasted 4.9 + 1.7 µg/mL vs $C_{max/ss}$ fed 5.9 + 1.7 µg/mL). These results indicate that concomitant food intake can alter theophylline bioavailability from Tromphyllin. It should be noted that differences in C_{max} are often dampened by comparing profiles at steady state, thus the C_{max} difference would be expected to be more pronounced in a single-dose study comparison (the situation corresponding to the in vitro studies).

Although a direct comparison of the pharmacokinetics of the two HPMC formulations has not been reported, it is reasonable to assume that administration immediately after a high-fat breakfast would result in markedly different plasma levels, whereas they should generate very similar plasma levels when given in the fasted state.

SUMMARY

For theophylline MR products in general, it appears that release rate depends more on formulation variables (excipients, coating, drug loading, matrix, etc.) than on the type of formulation per se (single- vs multiple-unit). The results presented here indicate that the therapeutic effect is likely to be influenced by the dosing conditions; they further illustrate the importance of choosing suitable in vitro test conditions. In particular, the importance of simulating gastrointestinal composition and transit conditions in both fasted- and fed-state when testing extended-release dosage forms was demonstrated.

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Appendix

Generation of Biorelevant Media Profiles for Prediction of Food Effects on Release from MR Dosage Forms

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INTRODUCTION

The in vitro simulation of gastrointestinal conditions with respect to composition and transit is crucial to forecasting drug release from modified-release (MR) dosage forms under different dosing conditions. The use of a biorelevant "pH-gradient" using variations on the composition of FaSSIF and FeSSIF in USP Apparatus 3 (Tables 3 and 5 of the main manuscript) provides good predictions of the in vivo behavior of MR formulations (*1–3*). However, FaSSIF and FeSSIF (*4*) have to be freshly prepared on the day of the experiment. In terms of creating a biorelevant pH gradient, this would become a cumbersome procedure because three different types of FaSSIF and FeSSIF media need to be prepared for each experiment. Based on these considerations, a methodology that enables the preparation of media in an easy and efficient manner was developed.

MATERIALS AND METHODS

The concept was to design a concentrate from which the different FaSSIF and FeSSIF compositions could be prepared. Reproducibility of media composition in terms of bile salt concentration, pH, volume, and osmolality was also considered.

Materials

Taurocholic acid sodium salt (PCA code 2012, lot # 20000060181) was purchased from Tiefenbacher (Hamburg, Germany). Egg lecithin (Lipoid E PC, lot # 105026-1) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All other compounds were of analytical grade or equivalent and purchased commercially.

Methods

The new method of media preparation was established as follows:

(1) A concentrate of bile salts in blank FaSSIF or of FeSSIF was prepared.
(2) The concentrate was diluted with blank FaSSIF or FeSSIF.
(3) The pH value was adjusted using a sodium hydroxide solution specifically designed for this purpose.
(4) The volume was adjusted to the desired value.

Using this method, reproducible media are easy to prepare in a short time. Furthermore, the concentrates can be used for the preparation of dilutions for 0–3 weeks when stored at 4–8 °C (unpublished data). In the subsequent section, the scheme for preparing quantities of FaSSIF and FeSSIF media that are required for one Bio-Dis run (n = 6, 200 mL of media per vessel) is illustrated.

Preparation of the FaSSIF Gradient

First, blank FaSSIF was prepared by dissolving 1.74 g of NaOH, 19.77 g of $NaH_2PO_4 \cdot H_2O$, and 30.9 g of NaCl in 5 L of purified water and adjusting the pH to 6.5 with 1 N NaOH or 1 N HCl (*1, 2*). Then, the FaSSIF concentrate for the preparation of the different types of FaSSIF media was prepared by dissolving 6.6 g sodium taurocholate in 500 g of blank FaSSIF. To this solution, 2.36 g lecithin was added. The resulting mixture was covered and stirred for 4 h at ambient temperature until a clear or slightly opaque solution was obtained. Finally, the weight was brought to 1,000 g (1 L) with blank FaSSIF. Compared with regular FaSSIF, the resulting FaSSIF concentrate contains a 4-fold higher concentration of bile components.

In the next step, a 1 N sodium hydroxide solution was prepared to adjust the pH. Besides sodium hydroxide, this solution contained sodium dihydrogenphosphate and sodium chloride in concentrations that correspond to those in blank FaSSIF. It is not necessary to prepare this sodium hydroxide solution every day; it can be stored at room temperature over several weeks, saving time in the preparation of FaSSIF-derived media. The detailed composition of the sodium hydroxide solution is given in Table 1.

Table 1. Composition of Sodium Hydroxide Solution for FaSSIF Media (Reproduced with permission from ref 3. Copyright 2005 Shaker-Verlag.)

1 N Sodium Hydroxide Solution for FaSSIF Media	
NaOH	40.00 g
$NaH_2PO_4 \cdot H_2O$	3.954 g
NaCl	6.186 g
Deionized water q.s. ad	1 L

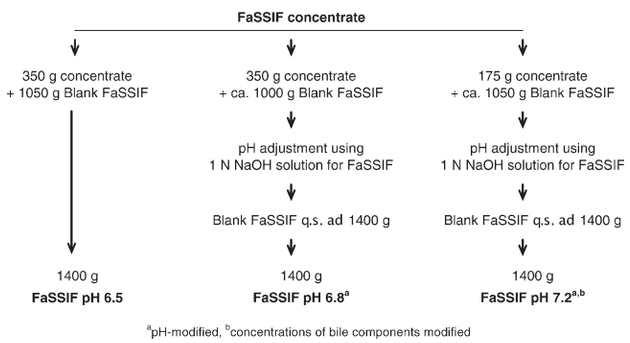

Figure 1. Preparation of different FaSSIF media from a concentrate.

The different FaSSIF media were prepared from the concentrate according to the following scheme. Because the density of both the concentrate and the resulting media is almost 1 g/mL, all additions and dilutions were made by weight.

Blank FaSSIF pH 6.8 and 7.2 were prepared from Blank FaSSIF pH 6.5 and "1 N sodium hydroxide solution for FaSSIF."

Preparation of the FeSSIF Gradient

First, blank FeSSIF was prepared by dissolving 20.2 g of NaOH, 43.25 g of glacial acetic acid, and 59.37 g of NaCl in 5 L of purified water and adjusting the pH to 5.0 with 1 N NaOH or 1 N HCl (*1*, *2*). Then, FeSSIF pH 5.0 was prepared by dissolving 16.5 g sodium taurocholate in 500 g of blank FeSSIF. To this solution, 5.91 g lecithin was added, and the resulting mixture was covered and stirred for 4 h at ambient temperature until a clear solution was obtained. Finally, the weight was brought to 2,000 g (2 L) with blank FeSSIF.

The concentrate for the preparation of the remaining FeSSIF media (i.e., FeSSIF pH 6.5 and FeSSIF pH 6.8) was prepared according to same scheme. Sodium taurocholate (19.8 g) was dissolved in about 500 g of blank FaSSIF to which 7.1 g lecithin was added, and the resulting mixture was covered and stirred for 4 hours at ambient temperature until a clear or slightly opaque solution was obtained. Finally, the total weight was brought to 1200 g (≈1200 mL) with blank FaSSIF. Compared with regular FeSSIF, the resulting concentrate is based on phosphate ions instead of acetate ions and contains twice the concentration of bile components. It is designated as "FeSSIF concentrate pH 6.5." An overview of the preparation of the two FeSSIF media from the concentrate is shown in Figure 2.

SUMMARY

Two schemes were designed to facilitate easy and efficient preparation of biorelevant "pH gradients" representing small intestinal conditions in the fasted and fed states. Results of release tests performed on theophylline MR preparations using these profiles correlated well with in vivo data (*3*). This indicates that the new schemes can be very useful in terms of performing

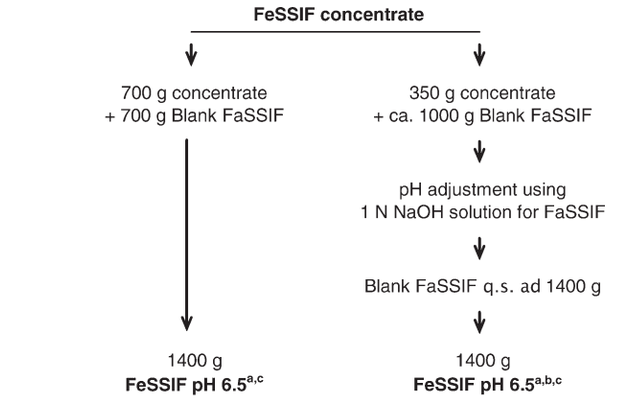

Figure 2. Preparation of two different FeSSIF media from a concentrate.

effective and reliable in vitro tests to predict the in vivo behavior of MR dosage forms.

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Editors Note: Dr. Klein is the originator of the method used in this article, which has been subsequently utilized by other authors (e.g., Samaha, D., et al. Modeling and Comparison of Dissolution Profiles of Diltiazem Modified Release Formulations. *Dissolution Technol.* **2009**, *16* (2), 41–46). The reference for the original method is Klein, S.; Stippler, E.; Wunderlich, M.; Dressman, J. Development of Dissolution Tests on the Basis of Gastrointestinal Physiology. In *Pharmaceutical Dissolution Testing*; Dressman, J., Krämer, J., Eds.; Taylor and Francis: Boca Raton, FL, 2005.

The FaSSIF and FeSSIF compositions were first published by Dressman et al., and an article by Marques (based on information from Dressman's lab) about practical preparation of the media was published subsequently (Dissolution Media Simulating Fasted and Fed States. *Dissolution Technol.* **2004**, *11* (2), 16). The original publication of FaSSIF and FeSSIF is Dressman, J. B.; Amidon, G. L.; Reppas, C.; Shah, V. P. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. *Pharm. Res.* **1998**, *15*, 11–22.