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Review Article

Theme: Role of Dissolution in QbD and Drug Product Life Cycle
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The Use of Biorelevant Dissolution Media to Forecast the *In Vivo* Performance of a Drug

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Abstract. Simulation of gastrointestinal conditions is essential to adequately predict the *in vivo* behavior of drug formulations. To reduce the size and number of human studies required to identify a drug product with appropriate performance in both the fed and fasted states, it is advantageous to be able to pre-screen formulations *in vitro*. The choice of appropriate media for such *in vitro* tests is crucial to their ability to correctly forecast the food effect in pharmacokinetic studies. The present paper gives an overview of the development and composition of biorelevant dissolution media that can be used for the *in vitro* simulation of different dosing conditions (fasted and fed states). In addition, the application of these media to predicting food effects is described in several case examples.

KEY WORDS: BA; biorelevant media; dissolution; fasted state; fed state; food effects.

INTRODUCTION

Dissolution testing was first established more than half a century ago and for many years was mainly performed to address questions of quality control. However, in recent years, there has been a strong push to identify bioavailability (BA) problems of a drug formulation based on the results of appropriately designed dissolution experiments. Thus, the scope of dissolution testing has expanded considerably to include screening formulations and predicting the *in vivo* performance of drug formulations. To answer questions in terms of the BA of oral drug formulations, it is crucial to run dissolution tests under conditions that closely resemble the key parameters of human gastrointestinal physiology. In addition to the choice of adequate equipment and appropriate instrument parameters, the use of physiologically relevant dissolution media is of great importance. Many of the dissolution media described in international pharmacopoeia are not adequate for this purpose. During the last decade, biorelevant media have been developed to simulate conditions in the stomach and small intestine before and after meals. In the present paper, the composition of these media and examples of their application to predicting food effects from *in vitro* studies will be described. Particular attention will be given to poorly soluble compounds as it often can be observed that their BA is highly dependent on the dosing conditions.

DRUG DISSOLUTION AND BIOAVAILABILITY

Drug dissolution in the physiological environment of the GI tract is the primary step in the oral absorption process from a pharmaceutical dosage form. Since only dissolved drug can permeate the mucosa at the absorptive sites in the GI tract (1), both the solubility of the drug and its dissolution rate are crucial for its *in vivo* behavior.

First theories on the dissolution process were described more than a century ago by Noyes and Whitney in 1897 (2) and extended by Nernst and Brunner in 1904 by applying Fick's law of diffusion (3). From the following equation, based on Nernst–Brunner and Levich modifications of the Noyes–Whitney model (4,5), the factors important to the rate of drug dissolution (DR) can be identified:

$$\mathrm{DR} = \frac{\mathrm{d}X_\mathrm{d}}{\mathrm{d}t} = \frac{A \times D}{\delta} \times \left[\frac{C_\mathrm{s} - X_\mathrm{d}}{V}\right]$$

where A is the effective surface area of the drug, D is the diffusion coefficient of the drug, δ is the effective diffusion boundary thickness adjacent to the dissolving surface, C_s is the saturation solubility of the drug under luminal conditions, X_d is the amount of drug already in solution, and V is the volume of the dissolution medium.

Some of these parameters are mainly determined by the physicochemical properties of the drug itself, but several are strongly affected by the conditions in the gastrointestinal tract (4) and also by the composition of the dosage form. The composition of the gastrointestinal content may significantly

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influence drug solubility. For lipophilic drugs, fat level and bile salt concentration have been shown to be the most pertinent factors. In the case of ionizable drugs, buffer capacity and pH are also relevant to the dissolution rate (6). For formulations containing highly soluble drugs, these parameters play only a subordinate role. However, they can be crucial in limiting drug release and BA of formulations comprising poorly soluble and/or ionizable drugs or key excipients. Thus, to predict the *in vivo* dissolution rate of such formulations based on *in vitro* dissolution data, it is particularly important to adequately simulate all parameters that may affect drug release from the dosage form in the dissolution experiment.

OBJECTIVES FOR IMPROVING THE BIORELEVANCE OF DISSOLUTION METHODS

To save time and costs associated with the need for pharmacokinetic and clinical studies, dissolution test systems capable of predicting BA by means of an *in vitro–in vivo* correlation would represent a valuable tool. Ideally, the methodology should be as simple as possible, reliable and reproducible, and make it possible to discriminate appropriately between different degrees of product performance (7). However, to achieve adequate predictability of the *in vivo* release behavior of a dosage form by use of *in vitro* dissolution data, physicochemical properties of the drug and its formulation as well as the relevant physiological conditions have to be considered in equal measure.

Official methods and regulations (8–10) predominantly prescribe the use of USP apparatus 1 (basket) and 2 (paddle) combined with aqueous buffer media. Several advantages are associated with using these apparatus. They are robust, easy to operate, and almost universally available. Moreover, there has been a wealth of experience with these methodologies and they are well accepted both by the user and the regulatory agencies. The paddle apparatus is commonly used for developing biorelevant dissolution test methods for immediate-release (IR) dosage forms. However, the simple aqueous buffers typically used in quality control laboratories are often not suitable for this purpose. Aqueous buffers can be used to reflect typical pH conditions in the stomach or small intestine, but do not represent other key aspects of the composition of the GI contents (e.g., osmolality, ionic strength, viscosity, surface tension) that can be relevant to drug release from the dosage form to be tested. In particular, they cannot be used to simulate the influence of food ingestion on drug release. In the case of poorly soluble compounds, it is often observed that the *in vivo* fraction absorbed increases when the drug is given with a meal. Thus, in order to simulate the effects of food on dissolution in the GI tract, it is equally important to develop representative dissolution tests for both the fasted and fed states.

MEDIA TO SIMULATE THE UPPER GI TRACT IN THE FASTED AND FED STATE

Assuming that the main differences in GI physiology between the fasted and fed states occur in the upper gastrointestinal tract and because IR formulations are expected to release drug in this region, most researchers have focused their efforts on developing tests to simulate dissolution under upper GI conditions. Over the past 10–15 years, biorelevant media to simulate both conditions in the stomach and small intestine before and after meals have been developed.

Compendial Dissolution Media

Simulated Gastric Fluid

The traditional medium to simulate gastric conditions in the fasted state has been simulated gastric fluid (SGF) of the USP. This medium contains hydrochloric acid and sodium chloride, as well as pepsin and water, and has a pH of 1.2. Although the medium addresses many of the qualities of gastric juice, there are some aspects that could be optimized. For example, most studies of gastric pH indicate that the across-the-board average gastric pH usually lies in the range 1.5–1.9 (4,11,12). For weak acids and neutral compounds, this small difference makes absolutely no difference in the dissolution characteristics, but for very poorly soluble weak bases, the dissolution results in compendial SGF are likely to overestimate the *in vivo* dissolution rate. Further deviations from gastric physiology are (1) the pepsin concentration, which is very high compared to that observed in gastric juice aspirated under fasted state conditions and (2) the surface tension of about 70 mN/m that does not take into account the much lower average surface tension of human gastric fluid, which has been repeatedly measured as lying in the 35- to 50-mN/m range (12–14).

Water

Water is an attractive medium that because of its simplicity has been widely used for quality control purposes. It could even be argued that it is physiologically relevant since many formulations are intended to be ingested with a glass of water. Furthermore, in those patients with hypochlorhydria (elevated gastric pH), due to aging and/or co-therapy with H_2 receptor antagonists and proton pump inhibitors, water may be a somewhat suitable medium as it roughly reflects the increased gastric pH and the low buffer capacity. However, the pH of water may vary with its source, and water has no buffer capacity. Thus, for the latter purpose, a better alternative, which would be more biorelevant in this context, is a diluted HCl/NaCl solution or a diluted acetate buffer with a final pH of around 5.

Simulated Intestinal Fluid

A frequently used medium for the simulation of small intestinal (SI) conditions in the fasted state is simulated intestinal fluid (SIF), a medium that was first described as a standard test solution in the USP more than 50 years ago. The only parameter that has been changed is the pH of the medium. As it was assumed that the pH in the small intestine is very close to blood plasma, the pH of SIF was initially set at 7.5. However, subsequent examinations of the pH in the intestinal tract (15) revealed that a pH gradient exists within the small intestine, that the pH becomes less acidic at more distal locations, and that pH values close to 7.5 can only be measured in the terminal ileum. As drug absorption during SI

passage is most efficient when drug release from the dosage form occurs at proximal SI sites, the more relevant pH values are those in the duodenum and the proximal jejunum. The use of an *in vitro* medium with an unsuitably high pH in contrast would most probably lead to false positive results, especially for poorly soluble, weakly acidic drugs and enteric-coated dosage forms. Thus, with USP 24/NF19 (16), the pH of the compendial SIF was revised to pH 6.8 (17), which can typically be measured in the mid-jejunum (18).

Compendial Media Simulating the Fed State

Currently, none of the guidance or international pharmacopoeias describes media to simulate food effects. Thus, water SGF and SIF are still the most commonly used dissolution media. However, as mentioned before, they do not take into account additional key parameters of the changing gastrointestinal environment after food intake and are therefore not useful to predict any food effects.

Biorelevant Dissolution Media

Fasted State Gastric Conditions: FaSSGF

Several attempts have been made to improve simulation of fasting conditions in the stomach. In most of these media, particular attention was given to the simulation of the surface tension measured in human gastric aspirates (5,19,20). However, in these media, non-physiologically relevant surface active agents, lower than physiological pH values or by far too high concentrations of pepsin or bile salts, were utilized. During almost 10 years of use, they were shown to often overestimate gastric dissolution because they induce solubilization effects greater than would be physiologically relevant (21). Recently, a fasted state simulated gastric fluid (FaSSGF) containing pepsin and low amounts of bile salt and lecithin was developed by Vertzoni *et al.* (22) (see Table I for composition).

Vertzoni *et al.* (21) compared the solubility of four poorly soluble drugs in human gastric aspirates and different kinds of simulated gastric fluids. In these experiments, they could clearly show that compared with data in other frequently used media, solubility data in FaSSGF provide a better basis for the assessment of intragastric solubility during a BA study in the fasted state. Thus, to better predict drug solubility and dissolution rate in the fasted stomach, the use of FaSSGF is strongly recommended for future *in vitro* experiments. Table I shows the composition for 1 l of FaSSGF. However, this volume is not representative of an average gastric volume in the fasted state. In the stomach, gastric juice secretion is usually low, but when co-ingested fluid (a glass of water) is taken into account, a more reasonable volume is in the order of 200–300 ml. However, with the standard paddle or basket apparatus, it is hardly possible to use such small test volumes without losing the reproducibility of the test. Thus, for volumes <300 ml, the "mini-paddle" apparatus, which has been established as a useful tool to performing reliable and reproducible dissolution tests (23,24), is highly recommended.

Table I. Sample Composition for Simulating Fasted State Gastric Conditions (FaSSGF)

FaSSGF pH 1.6		
Sodium taurocholate		80 μM
Lecithin		20 μM
Pepsin		0.1 mg/ml
NaCl		34.2 mM
HCl conc.	*qs ad*	pH 1.6
Deionized water	*ad*	1 l
pH		1.6
Osmolality (mOsmol/kg)		120.7 ± 2.5
Buffer capacity (mEq/pH/L)		–
Surface tension (mN/m)		42.6

Fasted State Small Intestinal Conditions: FaSSIF

By a series of tests in dogs fistulated at midgut, Greenwood (25) demonstrated that neither SIF nor another simple aqueous buffer have compositions similar to those found under fasted state conditions in the small intestine. As they do not adequately reflect all aspects of physiological conditions, dissolution rates of drugs in such media may not provide good predictions of the dissolution of drugs *in vivo*. In addition to pH, further important physiological factors not adequately addressed with these standard media are buffer capacity, bile and pancreatic secretion, surface tension, osmolality, and the volume of intestinal contents.

In response to these needs, attempts were made to create a biorelevant medium based on experimental data from the literature (19,26). Specifically fasted state simulating intestinal fluid (FaSSIF) was developed to simulate fasting conditions in the proximal small intestine. In addition to a stable phosphate buffer system that results in a pH representative to values measured from the mid-duodenum to the proximal ileum, this medium contains bile salts and phospholipids (lecithin). These compounds facilitate the wetting of solids and the solubilization of lipophilic drugs into mixed micelles. Thus, the dissolution of poorly soluble, lipophilic drugs may be enhanced considerably over the rate observed in simple aqueous solutions. Sodium taurocholate was chosen as a representative bile salt because cholic acid is one of the more prevalent bile salts in human bile (27–29). Furthermore, since the taurine conjugate has a very low pK_a, there is little likelihood of its precipitation or a change in the micellar size with minor variations in pH value within the typical range found in the proximal small intestine (pH 4–7). A suitable concentration of bile salt in the medium would be in the range of 3–5 mM. Lecithin is present in an approximately 1:4 ratio with the bile salt.

Buffer capacity data (25) indicate that buffer capacity in the fasted state is much lower than that in the fed state or that of SIF. For this reason, FaSSIF is only lightly buffered in comparison with SIF. From pharmacokinetic studies of drug absorption in the fasted state, it is known that by ingesting 200–250 ml of water with the dosage form, a maximum total volume of about 300–500 ml will be available in the proximal SI. Therefore, for dissolution tests, a volume of ≤500 ml is recommended to be consistent with the observed values (18). The detailed composition of FaSSIF is given in Table II.

According to Galia (30,31), FaSSIF is prepared as follows: 1.65 sodium taurocholate is dissolved in 250 ml blank FaSSIF (buffer without the bile components), 5.9 ml of a

Table II. Composition of the Biorelevant Medium used to Simulate Fasted State Conditions in the Small Intestine (FaSSIF)

FaSSIF pH 6.5		
Sodium taurocholate		3 mM
Lecithin		0.75 mM
NaH_2PO_4		3.438 g
NaCl		6.186 g
NaOH	*qs ad*	pH 6.5
Deionized water	*qs ad*	1 l
pH		6.5
Osmolality (mOsmol/kg)		~270
Buffer capacity (mEq/pH/L)		~12
Surface tension (mN/m)		54

solution containing 100 mg/ml lecithin in methylene chloride is added to form an emulsion, and the methylene chloride is eliminated under vacuum until a clear, micellar solution having no perceptible odor of methylene chloride is obtained. After cooling to room temperature, the volume is adjusted to 1 l with blank FaSSIF.

Based on the experience of the author, FaSSIF can also be obtained using a simplified method of preparation (24): 1.65 g sodium taurocholate is transferred into a 1,000-ml volumetric flask. Then 200 ml of blank FaSSIF are added. The flask is placed on a magnetic stirrer and the fluid is stirred until sodium taurocholate is completely dissolved (~2–3 min). Subsequently, 0.59 g lecithin is added and stirring is continued for about 4 h until a clear, micellar solution is formed. The volume is then adjusted to 1 l with blank FaSSIF.

Fed State Gastric Conditions: Milk and Ensure® Plus

In the fed state, the luminal composition in the stomach will be highly dependent on the composition of the meal ingested. Previous attempts to simulate typical postprandial gastric conditions include SGF, emulsions (32–34), complete nutrition products (32,33,35,36), and homogenized breakfasts (34). However, none of these media reflects all parameters that are important for determining food effects on drug release in the stomach. They either do not address the contribution of the meal to the composition in the stomach or represent pH values that are far from typical gastric pH values after meal ingestion, which often can be measured in the pH range of 3–6 or can even reach neutral pH values, depending on the meal composition. Moreover, these media are all not useful to reflect the changing pH and pepsin concentration over time that comes along with gastric acid secretion and digestion (18).

The ideal medium representing initial gastric conditions in the fed state should have similar nutritional and physicochemical properties to that of a meal, e.g., the standard breakfast recommended by the US FDA to studying the effects of food in BA and bioequivalence studies (37), have low inter-batch variability, and be easy to prepare. It should also be possible to manipulate the medium to simulate changes in the composition with time due to gastric secretion and digestion, if desired. Obviously, it is necessary to compromise some of these ideals to achieve a representative yet practical medium. Two alternatives that come close to these specifications are milk and Ensure® Plus (38). While milk was first investigated as a dissolution medium about 20 years ago (39–41), the use of Ensure® Plus has been established only a few years ago (38,42). Both standardized homogenized cows' milk with a fat content of 3.5% (whole milk) and Ensure® Plus have a similar composition to a breakfast meal with respect to the ratio of carbohydrate/fat/protein. Furthermore, their pH (6.5–6.6) and additional physicochemical properties are similar to those of homogenized and undigested standard breakfasts, whereas Ensure® Plus comes closer to the properties of the FDA breakfast (38) and milk is more useful when simulating a breakfast with a lower fat content. However, both media have also some of the same shortcomings as the aforementioned systems with respect to pH, pepsin, etc. In addition, as the stability of fresh milk at 37°C is a problem, heat-treated milk must be used. Furthermore, there may be variability in the composition of milk with source and season. Therefore, when comparing drug release from various formulations, it is important not to switch between different brands and qualities of milk.

Fed State Small Intestinal Conditions: FeSSIF

As in the stomach, conditions for drug dissolution in the proximal part of the small intestine are highly dependent on whether the drug is dosed in the fed or the fasted state. After ingesting a meal, there are changes in both the hydrodynamics and the intralumenal volume. The pH of the chyme after a solid meal is lower than the intestinal fluid pH in the fasted state, while buffer capacity and osmolality show a sharp increase (25). As well as these factors, the sharp increase in bile output could also be a major influence on the BA of a drug. In addition, specific interactions between the drug and ingested food components may occur. A dissolution medium for simulating the fed state small intestine should reflect all of these factors as close as possible. To better reflect the key parameters of the upper small intestine after meal ingestions, a fed state simulating intestinal fluid (FeSSIF), which at least partially meets these requirements, was developed about 10 years ago (26,30). The detailed composition of FeSSIF is given in Table III.

In order to achieve the higher buffer capacity and osmolality, while maintaining the lower pH value, representative of fed state conditions in the proximal small intestine, FeSSIF contains an acetate buffer. Taurocholate and lecithin

Table III. Composition of the Biorelevant Medium Used to Simulate Fed State Conditions in the Small Intestines (FeSSIF)

FeSSIF pH 5.0		
Sodium taurocholate		15 mM
Lecithin		3.75 mM
CH_3COOH		8.65 g
NaCl		11.874 g
NaOH pellets		4.04 g
Deionized water	*qs ad*	1 l
pH		5.0
Osmolality (mOsmol/kg)		~670
Buffer capacity (mEq/pH/L)		~72
Surface tension (mN/m)		48

are present in considerably higher concentrations than in the fasted state medium to reflect the biliary response to meal intake. Because of meal-induced secretions (43), a volume of up to 1 l would be reasonable for dissolution experiments simulating fed state small intestinal conditions (18).

According to Galia (30,31), FeSSIF is prepared as follows: 8.25 sodium taurocholate is dissolved in 250 ml blank FaSSIF (buffer without the bile components), 29.54 ml of a solution containing 100 mg/ml lecithin in methylene chloride is added to form an emulsion, and the methylene chloride is eliminated under vacuum until a clear, micellar solution having no perceptible odor of methylene chloride is obtained. After cooling to room temperature, the volume is adjusted to 1 l with blank FeSSIF.

As already mentioned for FaSSIF, the following simplified method can be used for the preparation of FeSSIF (24): 8.25 g sodium taurocholate is transferred into a 1,000-ml volumetric flask. Then 200 ml of blank FeSSIF is added. The flask is placed on a magnetic stirrer and the fluid is stirred until the sodium taurocholate is completely dissolved (~2–3 min). Subsequently, 2.95 g lecithin is added and stirring is continued for about 4 h (overnight, respectively) until a clear, micellar solution is formed. The volume is then adjusted to 1 l with blank FeSSIF.

Table IV. Test Media that Can Be Used for Solubility Profiling

Test media	pH	GI segment
Standard media		
SGFsp (USP)[a]	1.2	Stomach (fasted)
SGFsp mod.[b]	1.6/1.8	Stomach (fasted)
Acetate buffer	5.0	Stomach (hypoacidic)
Blank FaSSIF[c]	6.5	Upper small intestine (fasted)
Blank FeSSIF[c]	5.0	Upper small intestine (fed)
Acetate buffer[a]	4.5	Upper small intestine
SIFsp (USP)[a]	6.8	Mid small intestine
Additional media		
FaSSGF	1.6	Stomach (fasted)
Milk	6–7	Stomach (fed)
FaSSIF	6.5	Upper small intestine (fasted)
FeSSIF	5.0	Upper small intestine (fed)

[a] BCS conform test media
[b] pH-modified
[c] Contains no bile components
SGFsp simulated gastric fluid *sine pepsin*
SIFsp simulated intestinal fluid *sine pancreatin*
FaSSGF fasted state simulating gastric fluid
FaSSIF fasted state simulating gastric fluid
FeSSIF fed state simulating gastric fluid
Blank FaSSIF fasted state simulating gastric fluid without bile compounds
Blank FeSSIF fed state simulating gastric fluid without bile compounds

PREDICTION OF THE *IN VIVO* PERFORMANCE OF A DRUG FORMULATION—HOW TO PROCEED

The first step in choosing the right dissolution conditions for a drug formulation is to adequately characterize the solubility of the active pharmaceutical ingredient (API) itself.

Rather than a single point determination, a solubility profile of the substance in a set of test media representing physiological pH conditions in the human GI tract should be recorded to identify potential issues for drug precipitation *in vivo*. The solubility profile should be measured using a suitable and validated method, for example the shake-flask method, at 37°C to determine if the envisaged dose of the drug can be completely dissolved at all points of interest in the GI tract (44). A sample set of media that can be used for the solubility experiments is given in Table IV.

To classify the API according to the biopharmaceutics classification scheme (BCS) (45), this set of media also covers the three BCS conform test media required for solubility and dissolution experiments. For the solubility profiling of drugs formulated in IR formulations, conditions of the upper GI tract, which are listed in Table IV, are sufficient. For the characterization of drugs formulated in modified release dosage forms, additional media to simulate the lower small intestine (pH 7–7.5) and the proximal colon (pH 5–6) should be added (42,44).

Subsequent to the solubility experiments, the dose/solubility ratio (D/S) should be calculated according to the BCS. Whereas a D/S > 250 ml in aqueous media indicates solubility issues in the corresponding GI segment(s), a D/S < 250 ml at all pH values of interest indicates that dissolution is very unlikely to limit drug absorption. If the D/S is >250 ml but in the range of 250 to 1,000 ml in all aqueous buffers, solubility experiments should also be performed in biorelevant media (see "additional media" in Table IV). Particularly for lipophilic compounds where wetting problems can occur, physiological concentrations of bile salts may enhance wetting or may solubilize the drug and thus help overcome the solubility issues observed in simple buffers (44). For drugs having a D/S higher than 1,000 ml, bile components are unlikely to overcome all solubility problems. For such drugs, special formulation technologies are required. These can include material engineering like the micronization of the API, chemical modifications like salt formation, and a couple of other formulation strategies, e.g., solid dispersions, cyclodextrin complexes, or lipid formulations (46).

DISSOLUTION EXPERIMENTS

When solubility is identified as a critical parameter for the BA, the formulation strategies need to be evaluated and compared for their *in vivo* performance in humans. The starting point for formulation strategies is the dissolution test (46). As mentioned before, IR formulations containing highly soluble (BCS class I and III) drugs do not require very sophisticated dissolution setups and a simplified dissolution procedure can be followed. A good choice for this purpose is, e.g., the method stipulated by Stippler *et al.* (44,47) which is also part of the biowaiver dissolution method proposed by the WHO (48). In contrast, to predict the *in vivo* behavior of formulations containing BCS class II and IV drugs, such simple dissolution tests should not be the first choice. Since for BCS class II (poorly soluble but highly permeable) drugs the dissolution rate of the API is the rate-limiting step for drug absorption, the prediction of their *in vivo* behavior based on well-designed dissolution tests should be possible, meaning that the use of the biorelevant test media should lead to more predictive *in vitro* tests for drug dissolution and absorption in the small intestine. In the following case examples, the predictive power of simple buffer systems and

biorelevant dissolution media will be highlighted for a couple of formulations containing BCS class II drugs.

CASE EXAMPLES

Neutral Compounds—Danazol

Danazol is a synthetic steroid analogue of ethisterone which is used in the therapy of endometrioses, some benign breast disorders, and hereditary angioedema (49). The neutral drug is poorly soluble (aqueous solubility, 0.42 μg/ml at 37°C) (50), strongly lipophilic (log*P*, 4.53) (51), and shows a good permeability across GI membranes. Danazol is classified as a BCS class II substance, meaning that its oral BA is mainly determined by the solubility and dissolution rate in the upper GI tract (50). Because of its physicochemical properties and since danazol is non-toxic when given to male volunteers (52), it is often used as a model drug to study the impact of dosing conditions or different formulation technologies on the BA of a poorly soluble drug.

Because of the aforementioned reasons, danazol was considered as a suitable drug to study the predictive power of FaSSIF and FeSSIF in terms of *in vivo* of drug dissolution and subsequent absorption in the small intestine (30). Galia *et al.* studied the *in vitro* performance of tablets containing a single dose of 200 mg danazol using the paddle apparatus (100 rpm) and 500 ml of simulated intestinal fluid *sine pancreatin* (SIFsp), FaSSIF or FeSSIF. The resulting dissolution profiles shown in Fig. 1 clearly reflect the poor aqueous solubility of the drug since in SIFsp, no drug was released. In contrast, in the presence of bile components, an increase in dissolution of the drug was observed and the extent of drug release increased with rising concentrations of bile components. As a result, the total amount of drug released in FeSSIF was three to four times higher than in the corresponding volume of FaSSIF. Dissolution results indicate that the BA of danazol would be better when the drug is administered in the fed state. These observations are in good agreement with pharmacokinetic data available in the literature. In 1993, Charman *et al.* (53) have performed a study in 11 healthy female volunteers where they examined the effect of food on the BA of danazol. In this study, they observed that in contrast to administration in the fasted state ($AUC_{0-36\,h}$, 204±125 ng h ml^{-1}; C_{max}, 37±16 ng/ml), the BA of danazol increased by approximately fourfold when the drug was co-administered with food ($AUC_{0-36\,h}$, 639±259 ng h ml^{-1}; C_{max}, 101±42 ng/ml). Results from the biorelevant dissolution experiments are also in good agreement with *in vivo* results published by Sunesen *et al.* in 2005 (50) who performed a crossover study where patients received a capsule containing 100 mg of danazol either with water or with a lipid-rich meal. Similar to the Charman study, administration of danazol together with a lipid-rich meal increased the absolute BA significantly from 11.2±5.2% to 44±12%, which is also a fourfold increase when comparing fasted with fed state administration. From the results shown here, it can be concluded that the two biorelevant media, FaSSIF and FeSSIF, are a useful tool to predict the BA of the neutral BCS class II compound danazol.

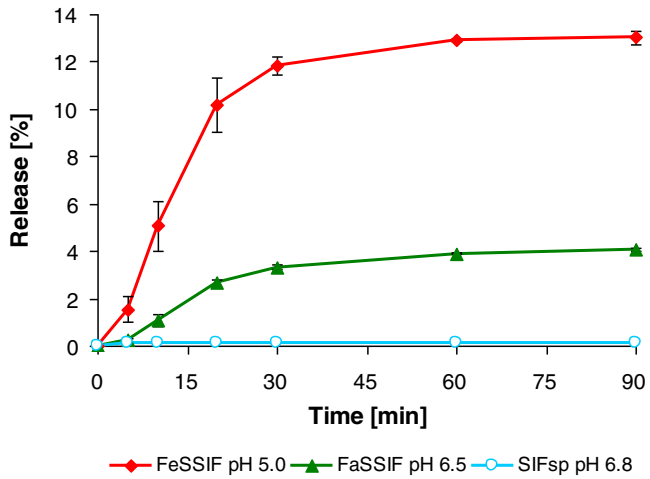

Fig. 1. Dissolution profiles of Danatrol® tablets obtained in media simulating the intralumenal composition of the small intestine before and after a meal (n=3±SD; adapted from Galia (30))

Weak Acids—Phenytoin

The dissolution of weak acids with poor solubility characteristics is somewhat more complicated than that of neutral compounds since the dissolution rate of these compounds is influenced by both the presence of surfactants that enhance wetting or solubilize the drug and the pH in the different sections of the GI tract (44). Thus, the dissolution performance of the API reflects an interplay of various factors which makes prediction of the *in vivo* dissolution behavior more difficult. Certainly, the fasted stomach represents the least favorable place for weak acids to dissolve. In contrast, many of these compounds dissolve quite well when moving to more neutral conditions in the small intestine.

Phenytoin is a hydantoin antiepileptic with anticonvulsive and antiarrhythmic properties. The drug is a lipophilic (log*P*, 2.47), poorly soluble weak acid (pK_a, 8.3) (49) and has been classified as a BCS class II drug (54). For this reason, the rate and extent of phenytoin absorption is mainly determined by the rate and extent of dissolution in the GI tract. To date, the best *in vivo* performance is obtained after administration of the micronized sodium salt of phenytoin. However, most of the marketed oral IR formulations still contain the unmodified drug. Several of these phenytoin IR formulations were examined for their *in vitro* dissolution behavior in compendial and biorelevant media. Figure 2 shows the release profiles obtained from an IR tablet formulation in compendial and biorelevant dissolution media.

Results from the dissolution studies revealed that drug release from both the pure drug (data not shown here) and the marketed formulation was independent on the composition and pH of the compendial test media (12–20% of drug release over 4 h). However, dissolution profiles generated with biorelevant media indicate that drug release, particularly from formulations containing the non-micronized drug, is affected by the presence of bile components. Comparison of dissolution profiles obtained in blank FaSSIF and FaSSIF and blank FeSSIF and FeSSIF indicates that bile components play an important role in the solubilization of phenytoin (~36% of drug released in FaSSIF and ~50% in FeSSIF over 4 h). FeSSIF significantly increased AUC_{0-t} by ~45% from the

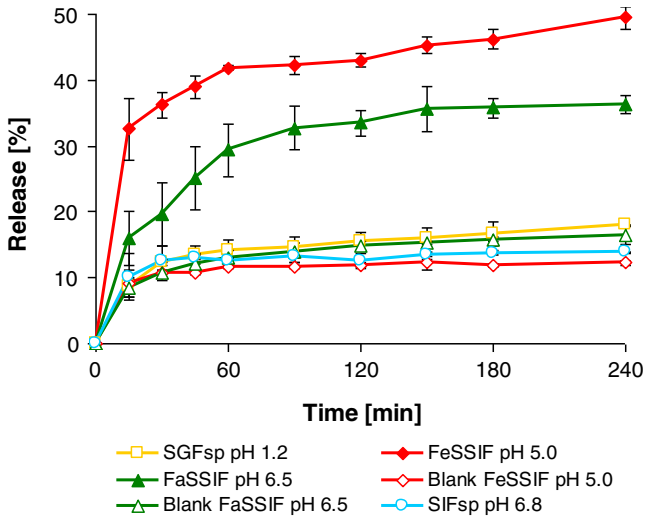

Fig. 2. Dissolution profiles of Phenhydan® tablets obtained in compendial and biorelevant media simulating the intralumenal composition of stomach and small intestine before and after a meal (n=3±SD)

marketed preparation. The results from the dissolution studies shown here were in good agreement with data from an *in vivo* study performed in New Zealand white rabbits (55) where an increase of 41.7% in $AUC_{0-\infty}$ was observed after administration of phenytoin in the fed state (with butter). The results are also in good agreement with two studies performed in human volunteers (56,57) where an enhanced absorption of phenytoin was observed when a phenytoin formulation was administered with food. The enhanced BA in the cited studies was most probably a result of both the presence of fat and digestive products in the GI lumen and the food-induced bile secretion on drug dissolution. In contrast to results obtained with biorelevant media, results with compendial media failed to match the *in vivo* data as they obviously do not reflect the essential conditions for the solubilization of poorly soluble drugs in the small intestine. Therefore, it can be concluded that for predicting the *in vivo* performance of weakly acidic lipophilic drugs, it is essential to not only focus on the changing pH conditions in the human GI tract but also to consider the impact on bile components on drug solubilization in the small intestine. FaSSIF and FeSSIF represent predictive dissolution media for this purpose.

Weak Bases—Itraconazole

In contrast to dissolution of weakly acidic drugs, the fasted stomach is the most favorable place for weakly basic drugs to dissolve. However, compared to the procedure for neutral and weakly acidic drugs, it is even more difficult to predict the *in vivo* behavior of poorly soluble weak bases. Due to their physicochemical characteristics, weakly basic compounds dissolve at very low pH values, and for such drugs, a huge inter- and intra-individual variability can be observed since even in the fasted state, gastric pH is rather variable than constant. Another point to consider is that the influence of pH on solubility is exponential, whereas, e.g., the impact of surfactants or fluid volume on solubility is linear (44). For this reason, already a modest change in gastric pH can create a tremendous change in solubility. Good case examples for subjects with elevated fasted gastric pH values are patients receiving proton pump inhibitors and H_2 antagonists or elderly people that suffer from hypochlorhydria. In such subjects, the median fasted gastric pH can be ≥5.0 and particularly in some of the patients with hypochlorhydria remains at this level even in the postprandial state (58). Although a very low gastric pH represents most favorable pH conditions, it does not always guarantee complete dissolution of a poorly soluble weak base in the stomach; the dissolution rate and extent of drug release can also be limited by wetting problems or a short gastric residence time of the formulation. Similarly, complete dissolution of the dose in the stomach does not equate with a good bioavailability. As only very few drugs can be absorbed from the stomach, the dissolved drug has to be emptied into the small intestine where it can be absorbed. However, exposure of the dissolved drug to the higher pH of the small intestinal fluids can result in precipitation of the drug before arriving at the site of absorption (44). This precipitation process can be influenced by various factors, e.g., the physicochemical characteristics of the drug itself, the gastric emptying rate, the pH in stomach and upper small intestine, the concentration of bile components, or the presence of digestive products. For these reasons, studying the dissolution behavior of a weak base only in SGF or even a whole set of compendial media is not sufficient to predicting what may happen *in vivo*.

Itraconazole is a triazole antifungal agent with a broad spectrum of activity. It is a highly lipophilic (log$P$, 5.66), weakly basic drug (pK_a, approx. 3.7) that was found experimentally to have very poor water solubility. Itraconazole can only be ionized and solubilized in aqueous media of very low pH. In various solubility studies of the pure drug, the amount drug dissolved at pH≥2 was below the detection limit (59). Similar observations could be made during dissolution experiments of the pure drug in the corresponding test media. In contrast, the solubility and dissolution rate of itraconazole could be tremendously improved by formulation with hydroxybutenyl-β-cyclodextrin (HBenBCD) (59). As shown in Figs. 3 and 4, it was possible to completely dissolve

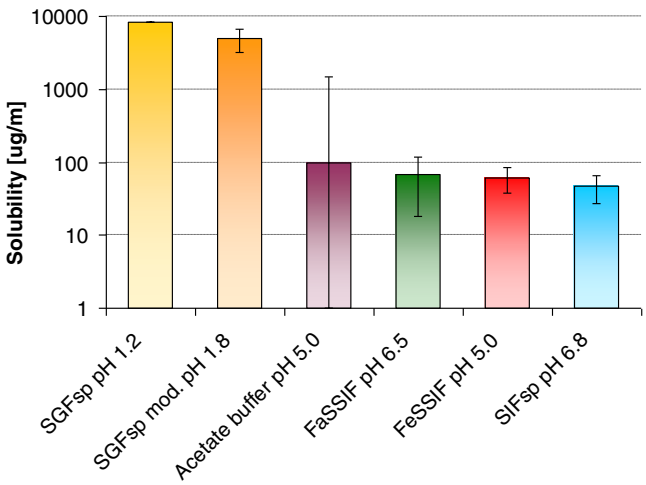

Fig. 3. Solubility data of itraconazole formulated with HBenBCD in compendial and biorelevant media

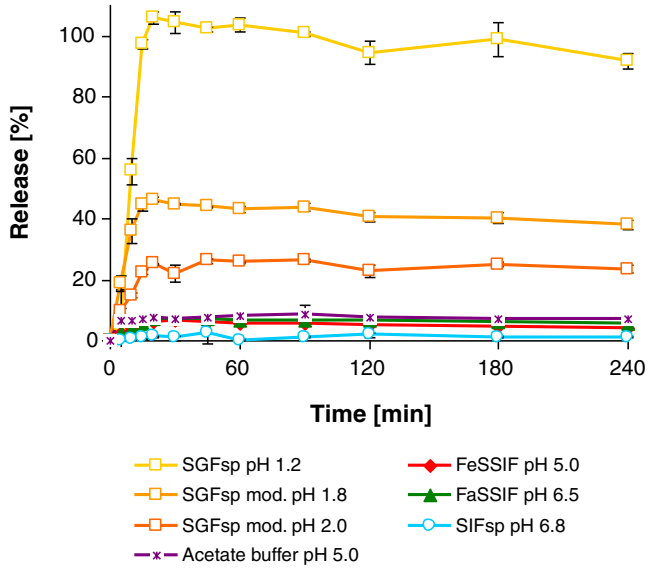

Fig. 4. Dissolution profiles of a itraconazole–HBenBCD complex obtained in compendial and biorelevant media simulating the intralumenal composition of stomach and small intestine before and after a meal (n=3±SD)

a 100-mg dose of itraconazole under conditions of the fasted stomach at pH 1.2. However, data obtained in these solubility and dissolution experiments also revealed the strong pH dependence of the drug's solubility and dissolution rate. A modest pH change from pH 1.2 to pH 1.8 or pH 2.0, two pH values lying in the physiological pH range of the fasted stomach, results in a sharp drop of the total amount of drug dissolved. In the dissolution experiment, only about 40% of the dose dissolved in 500 ml SGF at pH 1.8, and in SGF pH 2.0, the total amount of drug released declined to 25%. Further increase of the gastric pH to values corresponding to the average values measured in patients with elevated gastric pH (pH 5.0) resulted in another drop of the total amount of drug dissolved in the test medium (~8% of the dose dissolved in 500 ml). Dissolution experiments with marketed oral formulations of itraconazole followed the same trend (data not shown here).

Pharmacokinetic data published in the literature indicate that in humans, the absolute BA of marketed itraconazole capsule formulations is incomplete (<20%) (59,60). Furthermore, a pronounced food effect is reported for these formulations. Therefore, the dosage forms are recommended to either be taken with an acidic solution (e.g., Coke), to ensure dissolution of the drug at low gastric pH values, or together with a lipid-rich meal to enhance drug solubilization in the small intestine. In contrast to the food effects reported for the marketed formulations, the dietary state of the animals had no apparent effect on the observed oral bioavailability when the itraconazole/HBenBCD solid formulation was administered to male Sprague–Dawley rats in the fasted (AUC_{0-48h}, 6607 ng h ml^{-1}; C_{max}, 329.3 ng/ml; BA 20.2%) and fed (AUC_{0-48h}, 6,209 ng h ml^{-1}; C_{max}, 326.3 ng/ml; BA 19%) state (59). These observations are in good agreement with both results from solubility and dissolution experiments in biorelevant media. These experiments indicated that very similar amounts of the drug can be dissolved in FaSSIF and FeSSIF when itraconazole is formulated with HBenBCD and that therefore no food effects should be observed after oral administration of an itraconazole/HBenBCD complex. Overall, the study presented here clearly indicates the importance of resembling the physiological conditions relevant for drug dissolution and subsequent absorption as close as possible. Since itraconazole absorption from all marketed formulations is incomplete, it clearly becomes obvious that prediction of the oral drug absorption based on the dissolution results in simulated gastric fluid *sine pepsin* **(**SGFsp**)** pH 1.2 would overestimate the bioavailability of various formulations. In contrast, a prediction based on the results obtained in SIFsp pH 6.8 would underestimate the total amount of drug released *in vivo*. Dissolution media that reflect the relevant parameters *in vivo* more closely, e.g., pH-modified SGFsp (SGFsp mod. pH 1.8/2.0) and the biorelevant media FaSSIF and FeSSIF,

Table V. Composition of Fed State Simulated Gastric Fluid (FeSSGF) (61)

FeSSGF pH 5		
NaCl		237.02 mM
Acetic acid		17.12 mM
Sodium acetate		29.75 mM
Milk / acetate buffer		1:1
HCl conc.	*qs ad*	pH 5.0
pH		5.0
Osmolality (mOsmol/kg)		400
Buffer capacity (mEq/pH/L)		25

Table VI. Composition of the Updated FaSSIF (FaSSIF-V2) (61)

FaSSIF-V2 pH 6.5		
Sodium taurocholate		3 mM
Lecithin		0.2 mM
Maleic acid		19.12 mM
NaCl		68.62 mM
NaOH		34.8 mM
Deionized water	*qs ad*	1 l
pH		6.5
Osmolality (mOsmol/kg)		180±10
Buffer capacity (mEq/pH/L)		10

Table VII. Composition of the Updated FeSSIF (FeSSIF-V2) (61)

FeSSIF-V2 pH 5.8		
Sodium taurocholate		10 mM
Lecithin		2 mM
Glyceryl monooleate		5 mM
Sodium oleate		0.8 mM
Maleic acid		55.02 mM
NaCl		125.5 mM
NaOH		81.65 mM
Deionized water	*qs ad*	1 l
pH		5.8
Osmolality (mOsmol/kg)		390 ± 10
Buffer capacity (mEq/pH/L)		25

Addition of pancreatin (and $CaCl_2$) is optional

already discussed in the previous sections of the paper are much more useful for this purpose and therefore should be used already in the first stages of formulation development of weakly basic compounds.

FUTURE OUTLOOK

The first generation of biorelevant media was introduced about 12 years ago. These media were designed partly on the basis of literature data and partly on the basis of information on physicochemical parameters and composition of human GI fluids available at that time. However, already with the introduction of these media, it was well known that they do not account for all parameters that may be relevant to the dissolution rate of drugs and that they cannot reflect the changes in the intraluminal composition due to, e.g., gastric acid, bile, and pancreatic secretion over time.

Recent studies in healthy human volunteers have revealed that particularly conditions in the small intestine differ in some way from the composition of FaSSIF and FeSSIF (62). Therefore, updated compositions of biorelevant dissolution media, based on the physiological parameters recently summarized in the literature (62,63), were presented by Jantratid *et al.* (61). In the cited publication, particular care was given to media representing the intraluminal composition in the postprandial stomach and upper small intestine in the "early", "middle," and "late" phases of digestion. Thus, a series of so-called snapshot media was designed to reflect intraluminal conditions in these three digestive phases. In addition, updated general media for simulating postprandial conditions were devised. Compared to the first generation of media for the fed state, besides bile components, the updated media also contain digestive products that may contribute to the solubilization of a drug. In addition to the design of a series of new media representing fed state conditions in the upper GI tract, FaSSIF, the medium reflecting fasted state conditions in the upper small intestine, was "fine-tuned" to even better reflect this environment as well. The set of the new media named fed state simulated gastric fluid (FeSSGF; see Table V) and the version 2 of FaSSIF and FeSSIF (FaSSIF-V2 and FeSSIF-V2; see Tables VI and VII) is completed with FaSSGF, the composition of which is given in Table I. All background information on how the new media were developed as well as the detailed composition of the "snapshot" media should be taken from the original publication (61).

CONCLUSION

In the last decade, great progress has been made in predicting the *in vivo* performance of oral drug formulations based on well-designed dissolution tests. For poorly soluble drugs, prediction of *in vivo* performance would not have been possible without biorelevant dissolution media. For this reason, the first generation of biorelevant test media is now among the standard tools of many prefomulation laboratories. Recently, the second generation of biorelevant dissolution media has been reported in literature . As these updated compositions were designed on the basis of the most recent information on physiological parameters in the human GI tract, it is most likely that they will result in an even better predictive power than their precursers and therefore more extensively used in the coming years. In conclusion, modern formulation development is expected to rely heavily on biorelvant test media in the near and forseeable future.

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