Biorelevant Dissolution Methods and Their Applications in *In Vitro-In Vivo* Correlations for Oral Formulations

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Abstract: Dissolution tests that can predict the *in vivo* performance of drug products are usually called biorelevant dissolution tests. Biorelevant dissolution testing can be used to guide formulation development, to identify food effects on the dissolution and bioavailability of orally administered drugs, and to identify solubility limitations and stability issues. To develop a biorelevant dissolution test for oral dosage forms, the physiological conditions in the gastrointestinal (GI) tract that can affect drug dissolution are taken into consideration according to the properties of the drug and dosage form. A variety of biorelevant methods in terms of media and hydrodynamics to simulate the contents and the conditions of the GI tract are presented. The ability of biorelevant dissolution methods to predict *in vivo* performance and generate successful *in vitro–in vivo* correlations (IVIVC) for oral formulations are also discussed through several studies.

Keywords: Biorelevant media, stomach, small intestine, colon, hydrodynamics, dissolution, IVIVC, IVIVR.

1. INTRODUCTION

Oral drug administration remains the preferred route of drug administration with the highest degree of patient compliance and is the most user-friendly form of drug delivery; the majority (84%) of the 50 most-sold pharmaceutical products in the US and European markets are given orally [1]. For solid formulations, the dissolution process is one of the important limiting steps for the entire absorption process [2]. *In vitro* dissolution studies are an essential tool during the early and late stage drug development. The quality of the *in vitro* dissolution data are of great importance for their proper use in the evaluation of dosage form performance; the composition, volume and hydrodynamics of the contents in the gastrointestinal (GI) lumen following administration of the dosage form must be accurately simulated *in vitro*. One of the challenges of pharmaceutical research is correlating *in vitro* drug release information of various drug formulations to the *in vivo* drug profiles (IVIVC). The main objective of developing and evaluating an IVIVC is to reduce the number of bioequivalence studies performed during the initial approval process as well as with certain scale-up and postapproval changes. IVIVC development could lead to improved product quality and decreased regulatory burden [3].

Biorelevant media that simulate the conditions in the GI tract have been proposed for the characterization of the *in vivo* dissolution and solubility [4, 5] in the prediction of the absorption of drugs. These media are continuously updated as more physiological data become available [6]. Biorelevant dissolution testing designed with appropriate simulated media and hydrodynamics are useful from the early stages of drug discovery and development for identifying the biopharmaceutical performance of the compound (i.e. solubility problems, food effect, precipitation in the small intestine) through the later stages of development to assist in formulation strategies and the establishment of *in vitro-in vivo* correlations that will lead to reduction of the number of animal experimentation, bioavailability and bioequivalence studies [e.g. 7, 8].

In the present paper the development of biorelevant methods and their applications in the prediction of plasma profiles and the development of IVIVCs are reviewed. The paper is divided in three main parts. In the first part, the development of dissolution media in order to simulate the pre- and postprandial states of the stomach, the small intestine and the colon is described. The second part deals with the description of compendial and biorelevant hydrodynamics used in dissolution experiments. At the last part applications of the biorelevant methods in the prediction of *in vivo* performance of oral formulations and the development of *in vitro-in vivo* correlations/relationships (IVIVCs/Rs) are presented.

2. BIORELEVANT DISSOLUTION MEDIA

For highly soluble compounds, dissolution is not rate limiting to oral absorption. In contrast to highly soluble compounds, for poorly soluble compounds, the choice of medium is expected to play a very important role in their dissolution. For such compounds, the dissolution may be influenced by a variety of factors such as pH, buffer capacity, ionic strength and solubilization effects due the presence of surfactants and/or food components [8].

In the fasted state, dissolution of poorly soluble non-ionizable compounds will be slow in the stomach and in many cases will not be complete before the compound

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1874-1266/10

reaches the first absorptive sites in the small intestine. For poorly soluble weak acids, dissolution during the gastric residence in the fasting stomach contributes little to the overall dissolution given that the small intestine with its higher pH contributes to fast and in many cases complete dissolution. Opposite to weak acids, poorly soluble weak bases administered in the fasted state are expected to be primarily dissolved during residence in stomach because their solubility is expected to be higher in the fasting stomach than elsewhere in the GI tract [2, 9, 10].

In the fed state, the gastric residence of solids is prolonged and, depending on the type of dosage form administered, it can last for up to several hours. Food components have been shown to delay the intragastric dissolution due to delayed tablet disintegration but, despite this delay, absorption usually continues to be controled by gastric emptying [11, 12]. The delayed intragastric dissolution in the fed state may affect the absorption rates of drugs (especially of poorly soluble compounds) and, subsequently can influence the rate of appearance in plasma compared to the fasted state [5, 11, 12]. In addition, the small intestine in the fed state provides an environment with a high solubilization capacity for poorly soluble compounds and may lead to enhanced oral bioavailability compared to fasted state.

Compendial dissolution media have been designed for quality control purposes and often fail for IVIVC of poorly soluble compounds because their composition does not take into account the physiological environment of the GI tract and the dosing conditions [7, 10, 13]. In an attempt to better predict *in vivo* performance of oral formulations biorelevant media have been proposed.

2.1. Media to Simulate Contents of the Stomach

Fasted State

Various media have been proposed for simulation of fasting gastric contents. The simplest dissolution medium is the Simulated Gastric Fluid (SGF) consisting of a hydrochloric solution with a pH of 1.2, containing pepsin (3.2 mg/ml) and having a surface tension approximately equal to water (~ 68 mN/m) [14]. In order to reduce its surface tension to physiological values, the addition of synthetic surfactants, like sodium lauryl sulfate (SLS) [2] or Triton-X [15] have been proposed (Table **1**). These media were shown to overestimate gastric dissolution because they induce solubilization effects greater than would be physiologically relevant [16]. Recently, Aburub *et al.* proposed a revised composition of SGF which contains lower amount of SLS (0.05 vs 0.25 %w/v) [17]. Nevertheless, further studies are needed to assure the *in vivo* predictiveness of the medium.

However, in these media, non-physiologically relevant surface active agents, lower than physiological pH values and/or high concentrations of physiological components were utilized. Artificial surfactants can interfere with salt formation rates of weak bases and, thus, dissolution can be affected in an artefactual way [18]. In particular, sodium lauryl sulfate hydrolyzes in solutions having pH lower than 4 [19] leading to inconsistent medium composition. Also, sodium lauryl sulfate interacts with gelatin at pH<5 making its use with gelatin capsule products problematic [20]. The potential components responsible for the low surface tension in the fasting stomach have not been conclusively identified. Although bile salt reflux into the stomach occurs sporadically, and the resulting amounts of bile salts in the stomach are very low compared with those in the small intestine, their presence in combination with the relevant amounts of pepsin appear to be the main factors relevant to the low surface tension in gastric fluids in the preprandial state.

In 2005, Vertzoni *et al*. [10] developed Fasted State Simulated Gastric Fluid (FaSSGF) (Table **1**) as a dissolution medium that reflects the actual gastric composition in the fasting state according to published physiological data. This medium has a pH of 1.6 and contains physiological relevant amounts of pepsin, bile salts and lecithin in order to obtain a surface tension close to that found *in vivo*. This medium appears to be more appropriate than the previously proposed media because it comprises only components that have been recovered from the fasting stomach.

Fed State

The contents of the fed stomach are complex, heterogeneous, and have physicochemical characteristics that change

Table 1. Composition of the Media to Simulate Gastric Contents in the Fasted State

	SGF_{SLS} a,b	SGF_{Triton} c	FaSSGF d
Sodium lauryl sulfate (%w/v)	0.25/0.05	-	-
Triton X 100 (%w/v)	-	0.1	-
Pepsin (mg/ml)	-	-	0.1
NaTc (μM)	-	-	80
Lecithin (μM)	-	-	20
NaCl (mM)	34.2	34.2	34.2
pH	1.2	1.2	1.6
Surface Tension (mN/m)	33.7	32.0	42.6
Osmolality (mOsmol/kg)	180.5±3.6	157.7±2.9	120.7±2.5

a: [2], b: [17], c: [15], d: [10].

with time [e.g. 21]. Homogenized long-life milk (3.5% fat), as a dissolution medium to simulate fed conditions in the stomach, was proposed more than 20 years ago [4, 22-29]. Liquid meals with physicochemical properties similar to those of meals typically administered in bioavailability / bioequivalence studies in the fed state have been also suggested for *in vitro* application [28, 30-32]. Milk and/or nutritional liquid products can be used only for simulation of initial gastric conditions in the fed state because the composition of the stomach contents in the fed state changes with time as secretions, digestion and gastric emptying proceed. pH in the stomach usually rises due to buffering effects of the meal contents, and may initially reach values of up to 7, depending on meal composition but with the continuous secretion of gastric acid, the pH value then trends back down to baseline over a period of several hours [5, 21, 33].

The changing intragastric composition could, thus, be taken into consideration with two approaches: either by using "snapshot" milk-based media [6], or by gradually digesting milk during the *in vitro* release test [11, 34].

Both concepts use homogenized long-life milk as the initial liquid medium. The lower nutrient content of milk as compared to nutritional liquid products makes it more representative of the intra-gastric conditions, where significant amounts of secretions (dilution of meal) occur. Also, the ratio of carbohydrate:protein:fat in milk is close to that observed in the stomach of healthy volunteers after the administration of meals typically administered in bioavailability/ bioequivalence studies in the fed state [21, 35].

The first approach is the development of "snapshot" media, each corresponding to a certain time-frame after ingestion of the meal [6]. Medium composition was designed in order to reflect the pH value, buffer capacity and osmolality of the gastric contents during the first 75 min (early), from 75-165 min (middle) and after 165 min (late) following meal ingestion. Table **2** summarizes the composition of these three "snapshot" media. These "snapshot" media can be used as sequential dissolution media in one test series (e.g. USP Apparatus 3 and 4). The "middle" medium (FeSSGF) is suggested as a representative of postprandial conditions for comparing formulations and/or predicting food effects compared with FaSSGF.

The second approach introduces the concept of gradual digestion of the meal during the dissolution experiment [34]. The initial medium is homogenized long-life milk (3.5% fat) which is gradually digested by adding physiologically relevant amounts of a hydrochloric solution (HCl 1.83 M) containing 1.1 mg of protein (pepsin) per ml into the vessel every 15 min from 0 to 90 min. Moreover, the simulation of gastric lipolysis can be achieved by adding two portions of lipase RN (lipase from *Rhizopus Niveus*) (at 0 min and 90 min after the beginning of the dissolution experiment) in order to maintain mean lipase activity levels between 20 and 50 U/ml [11, 36].

2.2. Media to Simulate Contents of the Small Intestine

Fasted State

The simplest medium representing conditions in the small intestine is Simulated Intestinal Fluid (SIF) [14] which has a pH of 6.8 and contains pancreatin. This compendial dissolution medium is primarily used for quality control purposes and cannot be expected to be suitable in all cases for *in vitro*/*in vivo* correlations [29]. For example, food effects on drug absorption cannot be forecasted since there is no distinction between fasted and fed state in the design of SIF.

In 1998, Dressman *et al.* introduced FaSSIF in order to simulate fasting state conditions in the small intestine according to physiological data [2, 4]. Apart pH, osmolality and buffer capacity, FaSSIF takes into account the solubilizing capacity of the intestinal fluids preprandially (Table **3**). FaSSIF has a pH of 6.5 and contains sodium taurocholate and phospholipids in a ratio of 4:1. Recently, Jantratid *et al.*, have updated the composition of FaSSIF [6] with only minor changes. The amount of lecithin is decreased from 0.75 mM in FaSSIF to 0.2 mM in FaSSIF–V2, osmolality is lower in FaSSIF-V2 and maleate buffer is used instead of phosphate buffer (Table **3**). Although, the principal buffer species in the fasted small intestine are the bicarbonates, media containing

Table 2. Composition of the Media to Simulate Gastric Contents in the Fed State [6]

	Early	Middle (FeSSGF)	Late
Sodium chloride (mM)	148	237.02	122.6
Acetic acid (mM)	--	17.12	--
Sodium acetate (mM)	--	29.75	--
Ortho-phosphoric acid (mM)	--	--	5.5
Sodium dihydrogen phosphate (mM)	--	--	32
Milk:buffer	1:0	1:1	1:3
Hydrochloric acid/sodium hydroxide	qs pH 6.4	qs pH 5	qs pH 3
pH	6.4	5	3
Osmolality (mOsmol/kg)	559 ± 10	400 ± 10	300 ± 10
Buffer capacity (mmol/l/ΔpH)	21.33	25	25

bicarbonates are not easy to be used, because they must be continuously sparged with carbon dioxide to maintain the desired pH, buffer capacity, ionic strength and osmolality [37]. Therefore buffers were selected primarily according to their ability to achieve the desired combination of pH, osmolality and buffer capacity [6, 38].

Table 3. Composition of the Media to Simulate the Contents of the Small Intestine in the Fasted State

	FaSSIF [a]	FaSSIF-V2 [b]
Sodium taurocholate (mM)	3	3
Lecithin (mM)	0.75	0.2
Dibasic sodium phosphate (mM)	28.65	-
Maleic acid (mM)	-	19.12
Sodium hydroxide (mM)	8.7	34.8
Sodium chloride (mM)	105.85	68.62
pH	6.5	6.5
Osmolality (mOsmol/kg)	270 ± 10	180 ± 10
Buffer capacity (mmol/l/ΔpH)	12	10

a: [4], b: [6].

Fed State

Compared to the fasting state, the contents of fed small intestine have higher concentrations of mixed micelles, as the gall bladder contracts in response to a meal and empty its contents into the duodenum [5]. Fed state simulating fluid (FeSSIF) was introduced together with FaSSIF in order to better reflect the postprandial environment in the small intestine [2, 4]. FeSSIF has a pH of 5.0, and as for FaSSIF, osmolality and buffer capacity were adjusted to be close to *in vivo* data (Table **4**). It is not easy to identify the buffer species in fed intestinal contents. Buffer species that can be generated by food digestion (e.g. amino acids) play an important role in maintaining the pH value but it is doubtful that the necessary buffer capacity could be achieved only with bicarbonate buffer as the typical pH value of the upper small intestine in the fed state is 5-6 [38]. FeSSIF contains higher concentrations of sodium taurocholate and phospholipids compared to FaSSIF and their ratio is kept to 4:1. Some adjustments to FeSSIF have been made and modified media have been proposed which are characterized as micellar solutions and/or bile salts-lecithin mixtures in order to better simulate the fed state in small intestine [39, 40]. Zangenberg *et al.* [41] developed a dynamic lipolysis model in order to simulate the digestion conditions in the small intestine postprandially. This model controls the rate of lipolysis by the continuous addition of Ca^{2+}. Such models could provide information about the transport of poorly soluble drug substances into the aqueous phase by solubilization in the micellar structures resulting from lipolysis [42].

Recently, Jantratid *et al.* [6] updated the composition of FeSSIF based on recent studies related to the characterization of the fed intestinal contents under conditions simulating bioavailability/bioequivalence [21] which indicated that the pH in the upper small intestine decreases rather slowly after meal intake [6]. To reflect the influence of digestion processes "snapshot" media were developed as for the fed state gastric media. "Early", "middle" and "late" phases represent different time-frames of the composition of the contents of the small intestine after the ingestion of a meal (Table **4**) [6]. In the updated media, the presence of lipolysis products (glyceryl monooleate and sodium oleate) in the fed intestinal contents was also taken into account. As with the biorelevant media simulating the contents of stomach, FeSSIF-V2 (Table **4**) is suggested as a representative of postprandial conditions in the small intestine for comparing formulations and/or predicting food effects as compared with FaSSIF-V2 [6].

Table 4. Composition of the Media to Simulate the Contents of the Small Intestine in the Fed State

	FeSSIF [a]	Early [b]	Middle [b]	Late [b]	FeSSIF-V2 [b]
Sodium taurocholate (mM)	15	10	7.5	4.5	10
Lecithin (mM)	3.75	3	2	0.5	2
Glyceryl monooleate (mM)	--	6.5	5	1	5
Sodium oleate (mM)	--	40	30	0.8	0.8
Acetic Acid	144	--	--	--	--
Maleic acid (mM)	--	28.6	44	58.09	55.02
Sodium hydroxide (mM)	101	52.5	65.3	72	81.65
Sodium chloride (mM)	173	145.2	122.8	51	125.5
pH	5.0	6.5	5.8	5.4	5.8
Osmolality (mOsmol/kg)	635±10	400 ± 10	390 ± 10	240 ± 10	390 ± 10
Buffer capacity (mmol/l/ΔpH)	76	25	25	15	25

a: [4], b: [6].

2.3. Media to Simulate Contents of the Colon

To date, the development of a medium that simulates the contents of the large intestine [43] was based on pH considerations without taking into account potential food effects because information on the conditions in this region of the intestinal lumen has been very limited. In particular, Fotaki *et al.* [43] developed Simulated Colonic Fluid (SCoF) based on pH values and short chain fatty acid concentrations according to physiologically available data. SCoF has a pH 5.8 and acetate buffer was selected to adjust the desired pH and buffer capacity (Table **5**).

Table 5. Composition of the Medium to Simulate the Colonic Fluid (SCoF) [43]

	SCoF
Acetic acid (mM)	170
NaOH (mM)	157
pH	5.8
Osmolality (mOsmol/kg)	295
Buffer capacity (mmol/l/pH)	29.1
Ionic strength	0.16

Recently, Diakidou *et al.* [44] characterized the contents of the ascending colon in healthy volunteers under fasting and fed conditions with a view to designing *in vitro* studies to explain/predict dosage form performance in the lower gut. This study showed that free water content, pH, buffer capacity, osmolality, surface tension, short chain fatty acid content and bile acid content are significantly affected by the dosing conditions applied during BA/BE studies, physicochemical characteristics that are of prime importance for dosage form performance. Based on these findings, work is currently underway to design the corresponding biorelevant media.

3. SIMULATION OF INTRALUMENAL HYDRODYNAMICS

Biorelevant dissolution testing improved the predictability of mean plasma levels where hydrodynamics employed in the *in vitro* dissolution setups were based mainly on compendial apparatus [7, 10, 45]. Hydrodynamics in *in vitro* experiments are reflected by the design of the apparatus, the agitation intensity, the flow and/or volume, the viscosity of the medium and practical issues, such as the position of the dosage form during the experiment.

The most common methods used for drug dissolution testing are the Apparatus 1 and Apparatus 2 (basket and paddle assembly, respectively) followed by Apparatus 3 (reciprocating cylinder) and Apparatus 4 (flow-through cell) [14]. Reynolds number can be used to assess the hydrodynamics when using these apparatus. The Reynolds number is a global, rough order—of magnitude estimate of fluid inertia (flow accelerations) to frictional force in the flow around the object [46]. Reynolds numbers for the bulk flow vary from less than 30 (USP Apparatus 4) [47] to more than 2000 (USP Apparatus 2) [48]. Currently, there is no data for USP Apparatus 3. Moreover, when using USP Apparatus 2 highly non-uniform shear rates have been observed and the flow exhibits large fluctuations in the velocity field, strong enough to displace tablets along the vessel [49]. Abrahamsson *et al.* reported that Reynolds numbers of the flow around tablets of 1 cm in diameter within the stomach are of order 0.01-30, most likely with occasional short periods of more rapid tablet motion and higher Reynolds numbers [46].

Apparatus 1 and 2 (referred to as closed systems) are relatively simple, robust, and adequately standardized. Permanent incubation of the dosage form in the same medium is taken place, but problems with homogeneity in the vessel, dispersion in the vessel with areas of high concentration, or agglomeration can appear. USP Apparatus 3 and 4 are appropriate for drugs and dosage forms where dissolution at multiple pH levels and/or sequential changes of media are needed to mimic *in vivo* dissolution (i.e. controled release dosage forms); the release experiments can be set up with a series of dissolution media in one single run. The differences in the residence times and the hydrodynamics in the different parts of the GI tract can be reflected by the "passage" of the dosage form in each medium and the applied flow/dip rates, respectively. Apparatus 4 can operate as either an open or closed system, which is important for the study of poorly soluble drugs. It can also be particularly useful for specific drug dosage forms (i.e. multiparticulate dosage forms, suspensions) as several cell types are available.

One of the first attempts to develop a biorelevant *in vitro* setup in order to predict *in vivo* disintegration times was at 1948 [50]. Apart from the gastric emptying process, this setup takes into account the amount and quality of saliva in the mouth, the time of swallowing, the acidity and volume of gastric juice at the time of swallowing, the amount of peristaltic movements and the hydrostatic pressure present during peristalsis [50]. From then, artificial GI systems for the study of digestion of foods were developed but without emphasizing in the simulation of intralumenal hydrodynamics [51, 52].

Information with regard to the hydrodynamics and mechanical environment of the gastrointestinal tract appeared in the literature recently [53-56] but it is still limited mainly due the complexity and variability of intralumenal motility [57].

Based on the analysis of movement patterns of the postprandial stomach, Abrahamsson *et al.* [46] developed an *in vitro* apparatus that can simulate the *in vivo* range of surface shear stresses relevant for the human stomach under fed conditions (Fig. **1**). The design provides a controled and predictable flow around the tablets and consists of a beaker (∅ 220 mm) with the tablet fixed on a steel wire. The beaker is glued to the centrally placed rod at the bottom so that when the rod is rotated at fixed revolution rate the beaker also rotates at the same rate. Using this apparatus shear force effects on drug release from matrix tablets relevant for fed state could be predicted but no direct correlation with data obtained from human stomach under fed condition has been shown. Nevertheless, using a similar apparatus which consists of the USP Apparatus 2 dissolution tester with the tablet

fixed on a steel wire intralumenal behavior of modified release tablets could be predicted [11, 58].

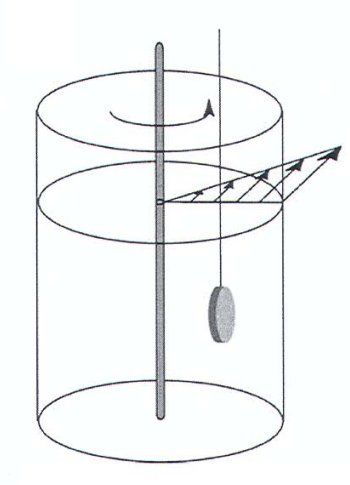

Fig. (1). Illustration of rotating beaker with a tablet fixed on a steel wire. The beaker is glued to the centrally placed rod at the bottom so that when the rod is rotated at fixed rpm the beaker also rotates at the same rpm (reproduced with permission from ref [46]).

Another apparatus that simulates conditions in the GI tract by applying forces to the dosage form has been developed by Burke *et al.* [59]. A programmable logic computer controls the frequency, duration and amount of force or compression that is applied to the dosage form. The dissolution device comprises a vessel containing the dissolution medium, an impeller, a sampler and a force application that has the dosage form housing and the force imparting mechanism.

Recently, a dissolution test apparatus that mimics the physical stress conditions during GI passage has been proposed (Fig. **2**) [60]. This device seems capable to simulate three mechanistic parameters which may be important for hydrodynamic and mechanical stress under *in vivo* conditions: discontinuous movement of the dosage form in the GI tract, pressure forces exerted by gut wall motility and interrupted contact of the dosage form to the liquid phase [60, 61]. Irregular absorption profiles in plasma after the administration of diclofenac extended release tablets could be predicted using this apparatus [60].

Although apparatus simulating the luminal hydrodynamics have started to appear in the literature, their ability to predict intralumenal drug release needs to be further validated.

4. PREDICTION OF *IN VIVO* BEHAVIOR AND DEVELOPMENT OF IVIVCS/RS

Data obtained with the most suitable dissolution model in terms of media composition and hydrodynamics have been used, in order to describe dissolution/release in various GI regions. Data from these studies have been used in model-dependent or model-independent approaches for the prediction of *in vivo* dissolution and absorption and the development of IVIVCs/Rs. In model-dependent approaches the dissolution profile or the dissolution rate constant are used in absorption modeling [softwares that simulate a complex system dynamically (i.e. Stella®) or physiologically based models of the gastrointestinal tract (i.e. GastroPlus™, PK-Sim®, Simcyp®, TNO integrated software) can be used]. In model independent approaches the amount dissolved/released over time or the *in vitro* dissolution/release rate are directly compared with the amount absorbed over time or the *in vivo* absorption rate [as calculated from deconvolution of the oral data- pharmacokinetic softwares (i.e. WinNonlin®, PCDCON®) can be used], respectively.

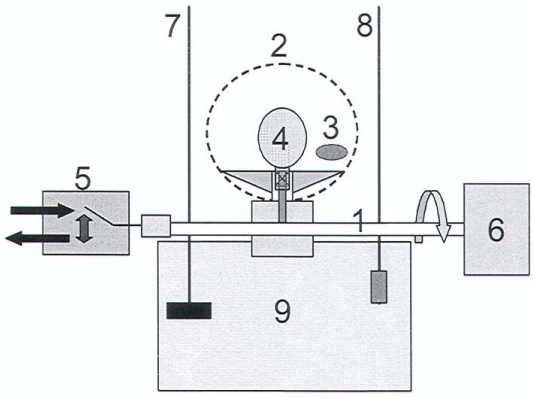

Fig. (2). Schematic representation of the dissolution stress test device (**1**) central axis (∅ 8 mm), (**2**) chamber (∅ 35 mm mesh size 0.5 mm, wire 0.1 mm), (**3**) dosage form, (**4**) inflatable balloon, (**5**) solenoid valves, (**6**) stepping motor, (**7**) stirrer (paddle 15×35 mm^2), (**8**) sampling, (**9**) standard vessel (reproduced with permission from ref [60]).

Biorelevant dissolution testing has been shown to be valuable through several studies. Food effect has been successfully predicted for poorly soluble drugs. For example, in the case of dipyridamole (a lipophilic weak base) the *in vitro* dissolution studies in biorelevant media showed that the dissolution rate of dipyridamole in FeSSIF (Table **4**) was higher than in FaSSIF (Table **3**) due to the presence of elevated bile salt and lecithin concentration combined with the lower pH value in FeSSIF; similarly, a higher oral bioavailability under fed conditions compared to fasting conditions has been observed in *in vivo* studies [62]. Furthermore, in the case that lipophilic weak bases are studied it has been shown that accurate *in vitro* simulation of the fasting gastric contents (FaSSGF- Table **1**) is crucial for the assessment of the absorption [10] (Fig. **3**). *In vitro* release experiments in biorelevant media (FaSSIF and FeSSIF data) predicted adequately a threefold increase in the absorption of danazol (a lipophilic, non-ionizable compound) that was observed *in vivo* when danazol was administered under fed state conditions compared to the administration under fasted state conditions [8]. *In vitro* experiments with modified biorelevant media in terms of bile salt and phospholipid levels for either fasted or fed conditions with the flow-through cell apparatus gave good IVIVCs for danazol under both simulated conditions [45] (Fig. **4**). Specifically, the physiologically most relevant correlation with *in vivo* results was achieved with a medium containing 6.3 mM bile salts

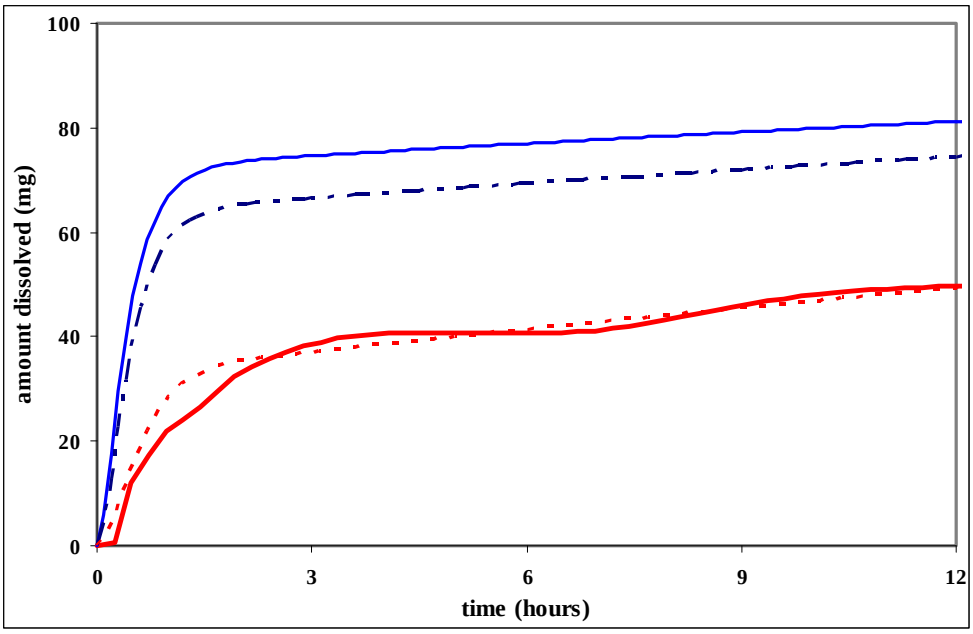

Fig. (3). Actual cumulative amount of GR253035X dissolved intralumenally after the administration of one GR253035X tablet in the fasted state vs. time (———) and simulated cumulative amounts dissolved intralumenally vs. time plots using data in SGF_{SLS} and FaSSIF (———), SGF_{Triton} and FaSSIF (— · — · —), and FaSSGF and FaSSIF (— — —) (reproduced with permission from ref [10]).

and 1.25 mM phospholipids and with a flow rate of 8 ml/min in the fasted state. In the fed state, an IVIVC could only be obtained by using the same flow rate and by including monoglycerides and fatty acids (lipolysis products) in a medium containing 18.8 mM bile salts and 3.75 mM phospholipids [45].

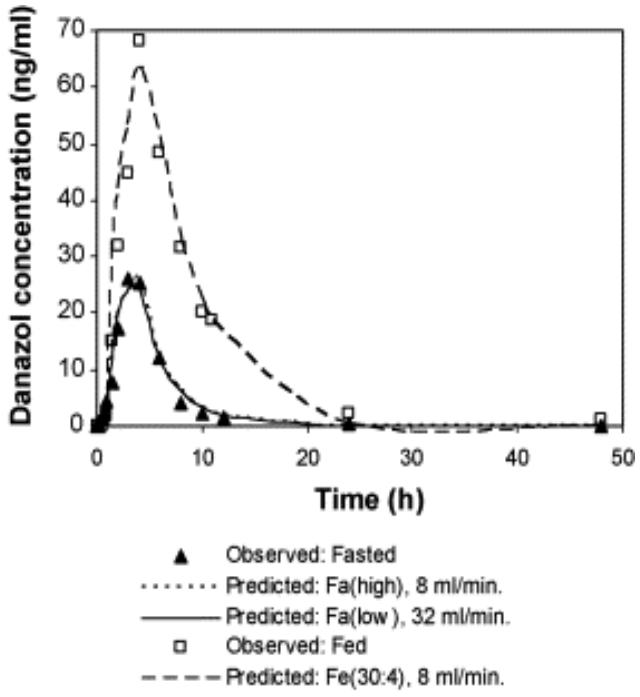

Fig. (4). Observed plasma concentration profiles of danazol in the fasted and fed states and predicted plasma concentration profiles based on flow through cell dissolution data (reproduced with permission from ref [45]).

In vitro dissolution testing using biorelevant media can be combined with in silico physiologically based pharmacokinetic (PBPK) modeling for the generation of simulated plasma profiles (model-dependent approach) and the development of IVIVCs. Following this approach, prediction of the plasma profile of orally administered lipophilic drugs was achieved based on *in vitro* dissolution data in biorelevant media in the cases that absolute bioavailability of the drug was known and the drug had dissolution limited absorption [7]. Recently, Shono *et al.* [13] have shown that the prediction of food effects on the absorption of celecoxib, a poorly soluble drug, could be achieved by this methodology too. *In vitro* experiments in biorelevant media with Apparatus 2 have shown that *in vitro* dissolution appears to be limited by the solubility of the drug combined with the fixed volume of media available, whereas *in vivo* the rate-limiting step to absorption seemed to be the dissolution rate due to the sink conditions generated by the high permeability of the drug. The use of the initial dissolution rates (in order to compensate for the closed system conditions) in biorelevant media in an *in silico* model (Stella® software) lead to successful predictions of the *in vivo* performance (Fig. **5**) [13].

In the case of controled release formulations, *in vivo* drug release occurs according to a specific predefined delivery pattern. Oral bioavailability is limited by intralumenal release (transit times, composition of contents of the GI tract, hydrodynamics) and these factors should be taken into consideration when trying to predict *in vivo* performance [63]. The use of a pH gradient and sequential changes of media (use of USP Apparatus 3 and 4) can be valuable during drug development as the formulations are exposed to the different conditions across the gastrointestinal tract, as discussed in Section 3.

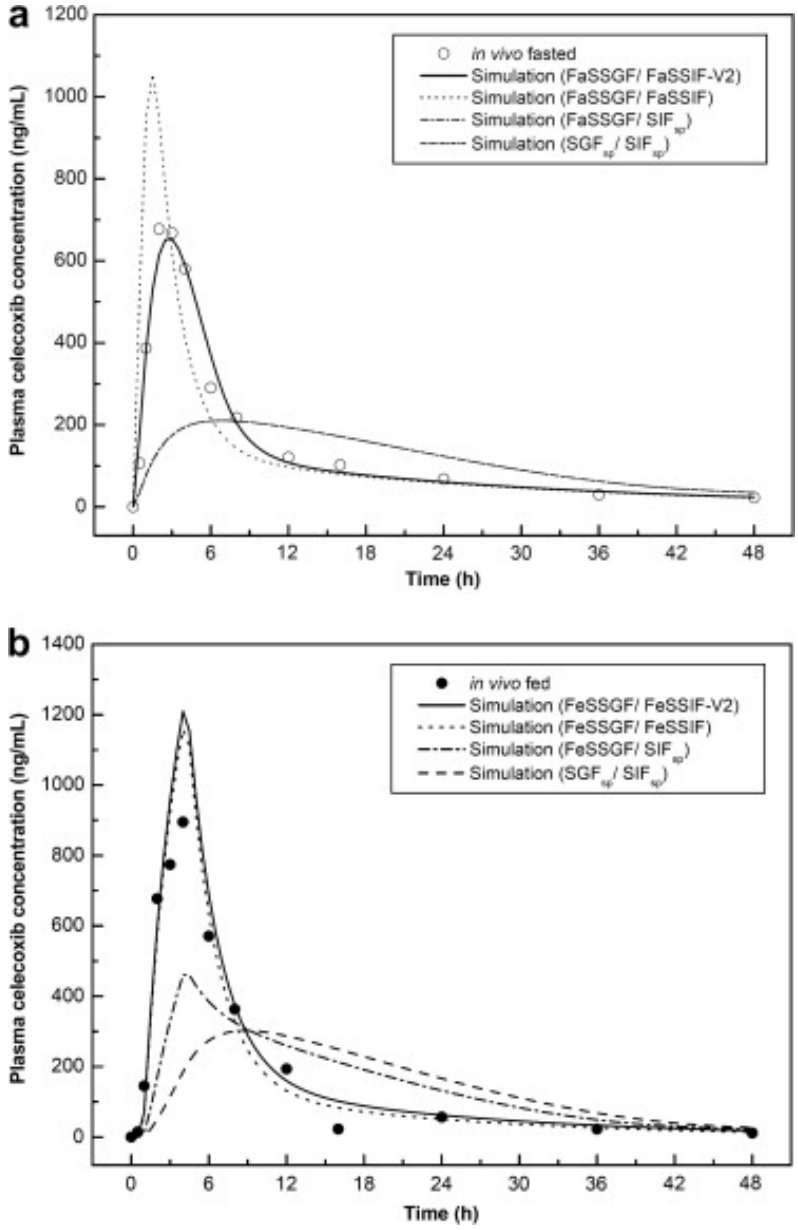

Fig. (5). Simulated plasma celecoxib concentration–time profile generated from combinations of dissolution media in (a) the fasted state and (b) fed state (reproduced with permission from ref [13]).

The use of the flow through cell apparatus with sequential changes of biorelevant media for the whole GI tract (stomach, small intestine, colon) under fasted conditions led to successful predictions of the absorption rates in humans for controled release formulations of isosorbide-5-mononitrate (ISMN) [BCS class I compound] (Fig. **6**) [43]. Physiological relevant flow rates and a model independent approach by direct comparison of the *in vitro* release rate with *in vivo* absorption rate (as calculated by deconvolution of the oral data) were used; *in vitro* flow rates were designed to achieve a balance between the total fluid volumes into which the formulation releases its contents intraluminally and the physiological residence times of the formulation taking into account the lack of radial water flux in the *in vitro* test system. In another example, when biorelevant dissolution data with the flow through cell apparatus was combined with reliable *in vitro* permeability data in a physiologically based pharmacokinetic model (Stella® software), a successful prediction of the absorption of levosulpiride (BCS Class III compound) housed in ER formulation was achieved [34, 64] (Fig. **7**).

The effect of the hydrodynamics of USP Apparatus 2, 3, and 4 in the development of IVIVCs for monolithic dosage forms (a BCS Class II compound housed in a hydrophilic matrix formulation and for a BCS Class I compound housed in an osmotic pump formulation) in biorelevant dissolution experiments in the fasted state was assessed [65]. Even though *in vitro* hydrodynamics affected the release profile from the hydrophilic matrix all three apparatus were equally useful in predicting the actual *in vivo* profile [65]. In another

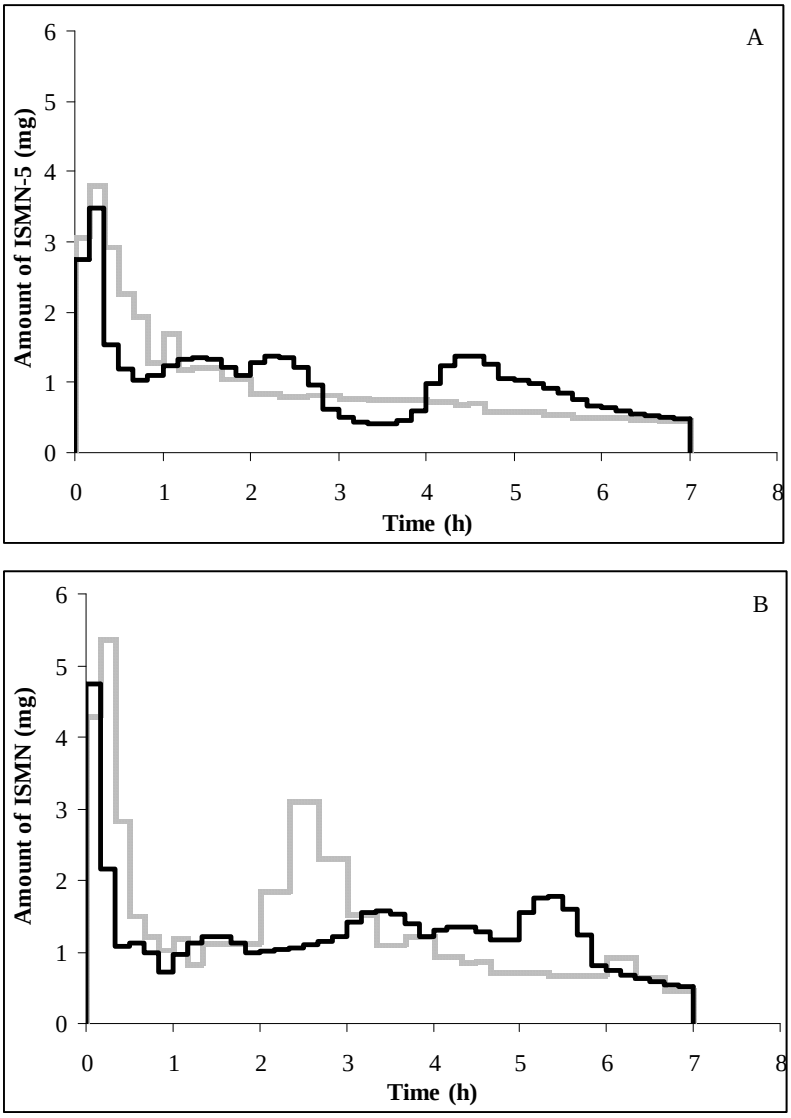

Fig. (6). Mean amounts of isosorbide-5-mononitrate (ISMN) absorbed in humans (——) (deconvoluted oral data) and released *in vitro* (⋯⋯) with the flow-through cell apparatus. (**A**) Imdur™; (**B**) osmotic pump (reproduced with permission from ref [43]).

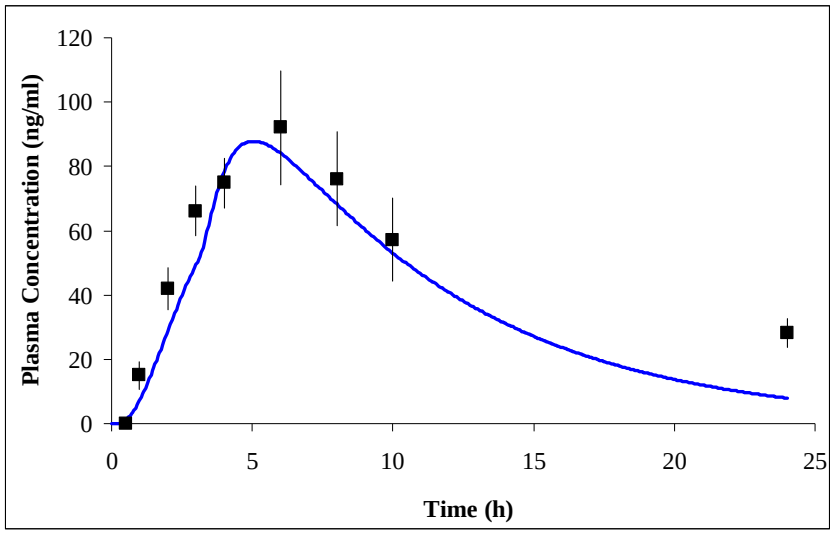

Fig. (7). Observed plasma levosulpiride concentration–time profile in the fed state and simulated generated from combination of dissolution data in the fed state and permeability data [64].

study, biorelevant dissolution testing with USP Apparatus 3 and 4 successfully predicted the food effect for modified release diclofenac sodium pellets, and the disadvantage of compendial dissolution tests (phosphate buffer with USP Apparatus 1 and 2) for this kind of prediction was also revealed [66]. Good correlations between *in vitro* drug release and *in vivo* drug absorption under both fasted and fed state conditions were achieved when the biorelevant dissolution test methods were used, with the flow-through cell method slightly superior to the Bio-Dis method. In a study of lipid formulations of a poorly soluble weakly acidic drug, biorelevant dissolution test methods with the Bio-Dis apparatus were superior to standard compendial methods with the paddle apparatus regarding their predictability of the *in vivo* performance of these formulations in dogs [67]. In this case, Bio-Dis method hydrodynamics facilitated release of the drug by emulsifying the formulation in the medium. Okumu *et al.* [68] showed the superiority of a dissolution test in modified biorelevant media with the flow through cell apparatus under a pH change protocol over dissolution tests in several media (modified biorelevant media and USP Simulated Intestinal Fluid) with the paddle apparatus under constant pH conditions in a study for the development of IVIVCs for montelukast sodium. The use of the *in vitro* biorelevant data with the flow through cell apparatus in a physiological based pharmacokinetic model in integrated software (GastroPlus™ software) led to successful prediction of the *in vivo* performance.

In some cases, the presence of lipolytic products in the dissolution media has been proposed in order to predict *in vivo* performance. For example, Diakidou *et al.* [11] showed the importance of intragastric lipolysis to felodipine release from a hydrophilic extended release tablet in the fed stomach. *In vitro* digestion and presence of biorelevant concentrations of lipase facilitated felodipine release from the eroded polymer, bringing the release profile closer to the *in vivo* intragastric data. The presence of lipolytic products in modified intestinal biorelevant media gave good *in vitro-in vivo* correlations for solid formulations of a poorly soluble compound and predicted successfully the pharmacokinetic parameters in dogs [69]. In this study the modified biorelevant media contained high levels of bile salts and lecithin as the intestinal bile salts levels in dogs have been reported to be higher than in humans.

5. CONCLUDING REMARKS

Biorelevant dissolution methods are used as an *in vitro* surrogate for *in vivo* performance. Biorelevant dissolution media that simulate closely the physiological *in vivo* conditions and *in vitro* hydrodynamic conditions that attempt to simulate the luminal hydrodynamics have been appeared in the literature. Appropriate *in vitro* conditions (media and hydrodynamics) that simulate the *in vivo* conditions can lead to successful predictions of the *in vivo* performance and *in vitro–in vivo* correlations (IVIVC) for oral formulations.

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Received: September 14, 2009 Revised: October 14, 2009 Accepted: November 02, 2009