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Research paper

In vitro models for the prediction of *in vivo* performance of oral dosage forms: Recent progress from partnership through the IMI OrBiTo collaboration

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A B S T R A C T

The availability of *in vitro* tools that are constructed on the basis of a detailed knowledge of key aspects of gastrointestinal (GI) physiology and their impact on formulation performance and subsequent drug release behaviour is fundamental to the success and efficiency of oral drug product development. Over the last six years, the development and optimization of improved, biorelevant *in vitro* tools has been a cornerstone of the IMI OrBiTo (Oral Biopharmaceutics Tools) project. By bringing together key industry and academic partners, and by linking tool development and optimization to human studies to understand behaviour at the formulation/GI tract interface, the collaboration has enabled innovation, optimization and implementation of the requisite biorelevant *in vitro* tools. In this paper, we present an overview of the *in vitro* tools investigated during the collaboration and offer a perspective on their future use in enhancing the development of new oral drug products.

1. Optimization of *in vitro* models: General principles for progress within the OrBiTo collaboration

In recent years, a plethora of biorelevant *in vitro* tools has emerged to better predict the *in vivo* performance of oral drug products; especially for formulations where the drug is poorly soluble, or where modified release is desired. Unlike dissolution methods used in quality control (QC) and batch release, where there is a high degree of standardisation in the methods used, these biorelevant tools have often been developed by a single commercial company or academic group and applied to just a few specific formulations. Therefore, within the work package 2 of the OrBiTo project, the greater challenge was not the development of new and even more biorelevant *in vitro* tools, but the optimisation, harmonisation and validation of tools that have already been described to some extent in the literature [1].

Key challenges for the *in vitro* tools in work package 2 of the OrBiTo project were therefore:

(a) how to optimise biorelevant *in vitro* tools in a more systematic way, and
(b) how to enable scientists to navigate their way through the multiplicity of tools and their variants available for prediction of dosage form behaviour.

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Within the collaboration, we had the unique opportunity to work with a large group of industry and academic partners, which enabled us to synergistically use data generated during the OrBiTo project and generate data on representative examples of oral products that were provided by industry partners. One key benefit of the collaboration was the direct availability of human investigational data generated by the *in vivo* tools work package (*i.e.* work package 3), which could then be used to optimise *in vitro* tools and compare *in vivo* data to the predictions made. Together, the combination of unique data sources and a wide range of expert collaborators helped us to address the first challenge above, and optimise and validate tools to an extent that would have not otherwise been possible. To address the second challenge, navigating the large and potentially confusing range of possible biorelevant *in vitro* tools that can be applied, we then developed a decision tree based upon the knowledge gained, with the aim of providing a guidance tool that will remain accessible after the OrBiTo project is completed. The decision tree, its development, and the vision for its use as a living document for inclusion of tools and additional knowledge beyond OrBiTo is described and briefly discussed in Section 4 [2].

In this review, we summarise the practical development, optimisation and validation of *in vitro* tools performed as part of the OrBiTo collaboration, with the work sub-divided according to the specific challenges the tools were designed to address.

2. Human data collected within the OrBiTo project to support the optimisation of *in vitro* setups

The key to the successful development of biorelevant *in vitro* tools used for the investigation of drug release and absorption from the gastrointestinal (GI) lumen are human reference data of high quality. These are critical to improving the physiological relevance and the predictive power of biorelevant *in vitro* testing. With respect to a proper simulation of GI conditions, accurate information on luminal fluid composition, transfer kinetics, GI motility and effects of food and fluid intake is required. Such information cannot be elucidated from the plasma concentration profiles that are often used to validate the predictive value of *in vitro* tools, but require more advanced methodologies such as aspiration of luminal contents, scintigraphy, telemetric capsules and magnetic resonance imaging (MRI). These approaches not only facilitate generation of data concerning the relevant physiological conditions in the human GI tract [3] but also allow for a more direct assessment of intraluminal drug and formulation behaviour. Within the OrBiTo project, multiple *in vivo* studies were performed in healthy volunteers to fill the gaps in our understanding of GI physiology and its interplay with oral drug delivery. The outcome of these studies has resulted in the generation of a unique database with various reference data that can be used to (i) validate *in vitro* tools and *in silico* models in their ability to predict intraluminal drug behaviour in humans, (ii) optimize experimental input variables of simulation tools, and (iii) identify the key processes to be simulated in any given scenario. Table 1 provides an overview of the *in vivo* studies performed within the OrBiTo project that aimed to further explore human GI physiology or the behaviour of various drugs and/or formulations within the human GI tract.

In several cases, these human data guided the development of *in vitro* simulation tools. For instance, the direct assessment of important

Table 1
OrBiTo *in vivo* studies that supported the development of *in vitro* tools by assessing physiological variables and drug behaviour in the GI tract of healthy volunteers.

Study	In vivo data obtained	Key use in development of in vitro tools	Refs.
Assessment of GI variables			
Telemetric capsule (SmartPill®)	• Pressure & pH profiles along GI tract in fasted and fed state	• Evaluating ability of *in vitro* dissolution tools (standard test apparatuses, dynamic open flow through apparatus) to simulate biorelevant pressure peaks	[4–6]
MRI studies	• GI fluid volumes and gastric emptying rates	• Highlighting the need to capture variability in GI fluid volumes and gastric emptying in simulation tools	[7]
Colonoscopic sampling of intestinal contents	• Physicochemical characterization of contents in distal ileum and cecum	• Optimization of biorelevant media and methodology to evaluate drug release and dissolution in the lower intestine	[8–10]
Assessment of GI drug behaviour			
Paromomycin (non-absorbable marker)	• Drug assessment in stomach, duodenum and plasma • Fasted and fed state	• Gastro-intestinal transfer rates for optimization of gastric emptying of drug solutions in *in vitro* tools (TIM, BioGIT)	[22]
Fenofibrate (micro- and nanoparticles)	• Drug assessment in stomach, duodenum and plasma • Fasted vs fed state	• Evaluating food-based solubilization requires dissolution testing combined with permeation assay • Validation of AMI-system in combination with dissolution testing • Validation of TIM	[19,20,70]
Posaconazole (suspensions and solution)	• Drug assessment in stomach, duodenum and plasma	• Validation of estimated duodenal concentrations in BioGIT system (supersaturation and precipitation) • Evaluation of AMI-system to capture the effect of supersaturation	[12,13]
Itraconazole (solid dispersion + cyclodextrin-based solution)	• Drug assessment in stomach, duodenum and plasma	• Validation of estimated duodenal concentrations in BioGIT system (supersaturation and precipitation)	[13,14]
Itraconazole (cyclodextrin-based solution)	• Drug assessment in stomach, duodenum and plasma • Impact of water intake (intraluminal dilution)	• Evaluating excipient-based solubilization requires dissolution testing combined with permeation assay • Validation of AMI-system in combination with dissolution testing	[20,52]
Albendazole	• Drug assessment in stomach and duodenum • Impact of supersaturation promoting excipients	• Validation of estimated duodenal concentrations in BioGIT system (supersaturation and precipitation)	[15,16]
Diclofenac (tablet of potassium salt)	• Drug assessment in stomach, duodenum and plasma • Fasted vs fed state	• Evaluating ability of USP II, dynamic open flow through test apparatus and TIMagc to simulate pH-dependent gastric and intestinal (re-)dissolution and precipitation, as well as food effect	[17]
Indinavir (capsule of sulphate salt)	• Drug assessment in stomach and duodenum • Fasted and fed state • Impact of PPI	• Ring study to evaluate interlaboratory variability in a USP II-based baseline dissolution test to assess intestinal precipitation	[18]
Ritonavir (solid dispersion)	• Drug assessment in stomach, duodenum and plasma • Fasted state • Impact of PPI	• Ring study to evaluate supersaturation and precipitation using several *in vitro* tools	Ongoing

physiological variables in the human GI tract contributed to the evaluation and optimization of the experimental conditions to be used in *in vitro* tools. In this respect, comparison of pressure recordings using a telemetric motility capsule (SmartPill®) in the GI tract of healthy volunteers with those in several *in vitro* dissolution tools revealed the inability of standard test apparatus to generate biorelevant pressure peaks [4–6]. A meta-analysis of MRI data from the human GI tract highlighted the substantial inter-occasion and inter-subject variability in fasted state gastric volumes and water emptying, both of which may substantially affect dosage form disintegration and drug release [7]. Thus, it was shown that prediction of drug product performance beyond the "average" administration conditions requires simulation tools that capture these sources of variability. Additionally, using colonoscopic sampling, the contents of the distal ileum and cecum in healthy adults were characterized under conditions simulating bioavailability and bioequivalence studies of drug products in fasted and fed state [8]. This information was then used to optimize biorelevant media and methodologies specifically designed to evaluate drug release and dissolution in the lower intestine [9,10].

Drug concentration profiling in the upper GI tract after administration of immediate release (IR) dosage forms to healthy volunteers [11] provided direct reference data to validate the concentrations simulated in dynamic dissolution tools, including the TNO Intestinal Models (TIM-1, tiny-TIM) and the Biorelevant Gastrointestinal Transfer (BioGIT) model. In this respect, intraluminal concentrations of the poorly water-soluble drugs posaconazole, itraconazole, albendazole and diclofenac helped demonstrate the predictability of supersaturation and precipitation events starting from different formulation strategies [12–17]. Data on the GI behaviour of indinavir [18] and ritonavir are currently being used in inter-laboratory variability tests across multiple OrBiTo partners (*i.e.* ring studies) to evaluate various tools for assessing supersaturation and precipitation. Intestinal profiling of the effect of food intake on fenofibrate absorption and the effect of cyclodextrins on itraconazole absorption identified the need to combine dissolution testing with assessment of permeation potential from luminal matrices to correctly judge the impact of food or excipient related solubilization on drug absorption [19–21]. Finally, the GI transfer of the non-absorbable marker paromomycin observed in healthy volunteers provided direct input to validate and optimize the GI transfer rate of drug solutions used in TIM and BioGIT [22].

The OrBiTo studies summarized in Table 1 exemplify the unique opportunities that arise when the development of *in vitro* tools is guided by reference data obtained directly from the human GI tract. Beyond the OrBiTo project, this approach should continue to be used to optimize *in vitro* tools for drug absorption, focusing on under-researched topics such as capturing variability, specific patient populations, and real-life dosing conditions.

3. Overcoming the limitations of pharmacopoeial setups – Recent advances from IMI OrBiTo

3.1. Use of standard pharmacopoeial setups with biorelevant media

Within an industry setting, standard dissolution apparatus described in pharmacopeia are widely available and commonly applied to drug release testing. Groups working on development/evaluation of oral formulations will typically have a good working knowledge of these models, especially the paddle apparatus, which is the most widely used. Therefore, one way to ensure that new *in vitro* tools are widely adopted is to develop biorelevant methods based upon these apparatus, including substituting biorelevant dissolution media for the simpler media typically used. This concept has been established since the 1990's when biorelevant dissolution media containing taurocholate and lecithin were first proposed [23]. Within the OrBiTo project, we have further explored the use of single and dual media paddle methods (USP apparatus II, ring study) [24] for the prediction of IR dosage form behaviour and the use of reciprocating cylinder methods (USP apparatus III)/flow through methods (USP apparatus IV) with biorelevant media for modified release dosage form behaviour [25–27].

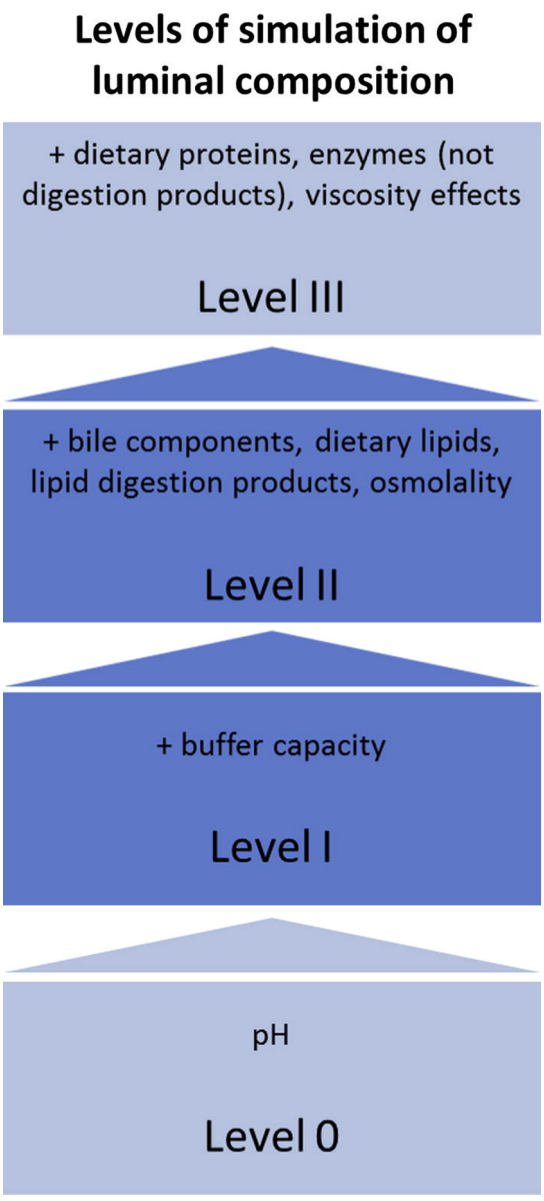


Fig. 1. The media complexity concept, adopted from Markopoulos et al. [28].

3.1.1. How complex do biorelevant media need to be? The "Levels" concept

One key concept established early in the collaboration, and especially relevant when using pharmacopeia-based setups is that of selecting a dissolution medium of an appropriate level of complexity. Markopoulos, Andreas et al. developed a "Levels" pyramid to guide dissolution scientists in the choice of media. Essentially, the "Levels" concept captures the principle that for optimal selection of a method for predicting *in vivo* performance, including *in vivo – in vitro* correlations/relationships (IVIVC/IVIVR), media complexity needs to be tailored to the properties of the drug and the formulation (Fig. 1). Examples ranging from drug/formulation combinations where simple buffers suffice (Level 0 media) all the way through to media that are needed to answer specific questions such as "What role does digestion play in drug release from the dosage form" (Level 3 media) [28] are provided (Table 2).

Table 2
Examples of levels of biorelevant media required for developing IVIVC or IVIVR for selected APIs and dosage forms (from Markopoulos et al[8], based on previously published data).

API	DCS classification	Type of dosage form	Level of biorelevant media required for IVIVC/R
Primaquine Phosphate	I	Immediate release tablets	0
Diclofenac sodium	I	Enteric coated pellets	1
Aprepitant	II/IV	Nanosized capsule formulation	2
Danazol	II (fasted state)	Lipid based formulations	3

3.1.2. New insights into simple pharmacopoeia-based setups for poorly soluble immediate release drug performance

Even with the simpler dissolution media used for batch release, reproducibility of data from one user/site to another can be problematic. Poor robustness or repeatability could potentially pose a significant barrier to greater use of biorelevant media in dissolution testing, especially if these data are to be used by industry in a regulatory setting. Media addition or transfer, which may be advocated in biorelevant dissolution testing to simulate stomach to intestine transit, additionally represents an increase in the level of complexity compared to the typical batch release dissolution test.

To address concerns that have been raised about the robustness and reproducibility concerns of biorelevant media, on the one hand, and two-stage testing, on the other hand, we conducted a series of ring studies. Sixteen consortium partners participated in two dissolution protocols. One involved dissolution in single media (FaSSGF and FaSSIF) and the other was a two-stage (dual media) test, in which the dosage form was first subjected to FaSSGF and then to FaSSIF. Several poorly soluble drugs in IR formulations were tested, using a paddle method at 75 rpm with 250–500 mL of biorelevant dissolution media [24]. Figs. 2a and 2b demonstrate the results for the single and dual media methods for zafirlukast tablets for the 16 partners involved in the ring studies.

When the data from the different partners were compared, the results showed excellent inter-laboratory reproducibility. Indeed, they were more reproducible between partners than previous ring studies performed on USP calibrator tablets [29,30]. In addition, there was no link between the batch of bile salt/lecithin powder used and the dissolution results, even when expired batches were used.

We anticipate that these data will encourage greater use of biorelevant dissolution media beyond their current, predominant use as an early stage formulation comparison tool in industry settings, as the

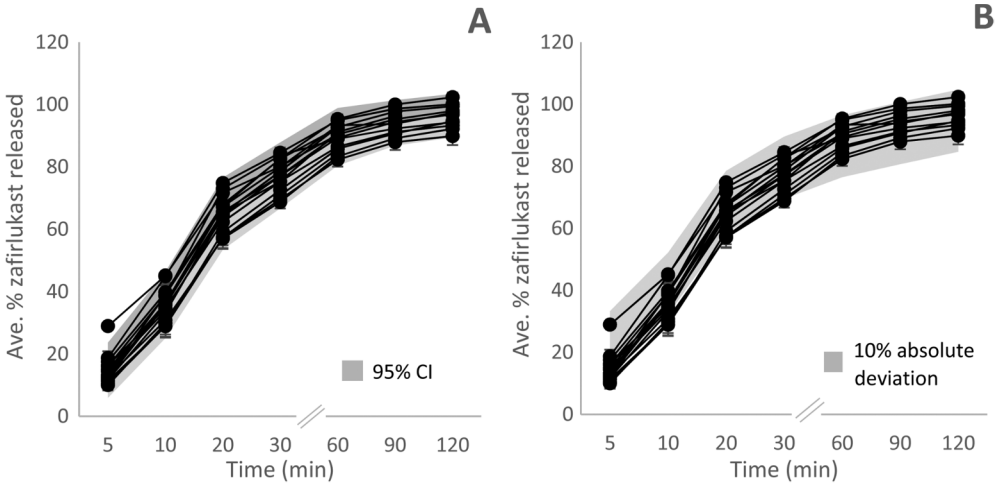


Fig. 2a. Individual mean results for dissolution of zafirlukast from Accolate 20 mg tablets in FaSSIF: panel A with the 95% confidence interval for the data shown in gray and panel B with an absolute deviation of 10% from the overall mean concentration profile shown in gray. Reprinted with permission from [24]. Copyright (2017) American Chemical Society.

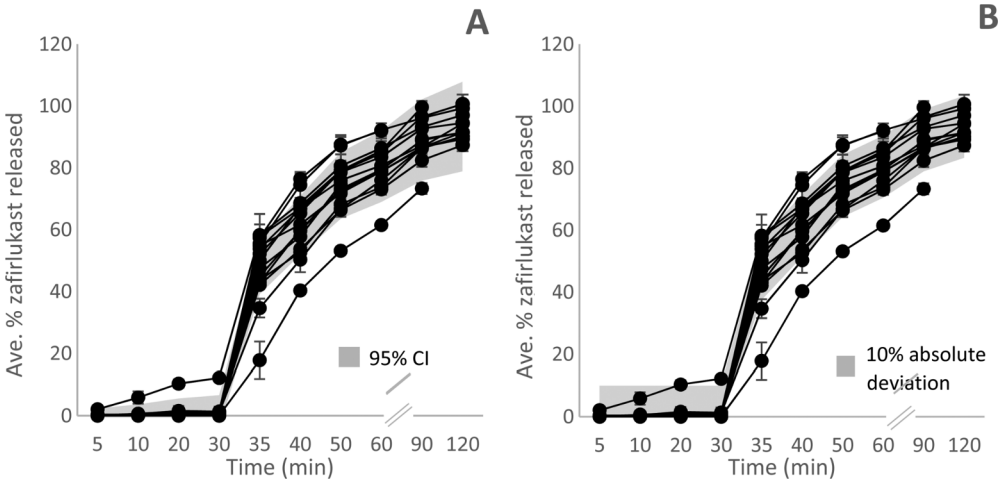


Fig. 2b. Individual mean results for dissolution of zafirlukast from Accolate 20 mg tablets in the two-stage dissolution test: with the 95% confidence interval for the data shown in gray (panel A) and with an absolute deviation of 10% from the overall mean concentration shown in gray (panel B). Reprinted with permission from [24]. Copyright (2017) American Chemical Society.

study dispelled a major perceived potential barrier to their application to dissolution testing carried out across multiple sites and/or in a routine testing environment.

3.1.3. New insights into simple pharmacopoeia-based setups for delayed release drug performance

In vitro biopharmaceutical evaluation of delayed release (enteric coated) solid dosage forms conventionally involves disintegration testing, as advocated in chapters USP 701 or PhEur 2.9.1, and dissolution testing (USP 711, Ph. Eur. 2.9.3) in a two-step procedure. For disintegration testing, the first step evaluates the resistance of the enteric dosage form against an acidic medium (0.1 N hydrochloric acid or simulated gastric fluid, SGF) for a defined duration. Subsequently, the disintegration is evaluated in simulated intestinal fluid (SIF USP) pH 6.8 or phosphate buffer pH 6.8 (Ph. Eur.) within 60 min (Ph. Eur.) or according to a specific product monograph (USP). These test procedures have been harmonized between USP and Ph. Eur. Although most marketed products fulfill these pharmacopeial requirements, there is convincing evidence that these test methods are not always biopredictive [31] and that the incidence of poor *in vivo* disintegration/dissolution is unusually high for enteric-coated solid dosage forms.

Within the OrBiTo project, a systematic study on improving *in vitro* test methods for such formulations was undertaken. A novel method based on adjusted phosphate buffer molarity was validated by using several enteric-coated aspirin formulations as well as by *in vitro – in vivo* correlation analysis. A key aspect of the method is to reflect not only pH changes going from the stomach to the small intestine but also the buffering capacity of the small intestine. This relatively simple *in vitro* method may improve prediction of post gastric emptying lag times and absorption rates of enteric-coated dosage forms [32].

3.1.4. New insights into simple pharmacopeia-based setups for extended release drug performance

The USP apparatus III (reciprocating cylinder) and USP apparatus IV (flow-through cell) both facilitate multiple media changes throughout an experiment and are therefore particularly suited to evaluating how a drug will be released from a modified release dosage form as it moves through the GI tract. Work performed in OrBiTo demonstrated that the optimal dissolution conditions and method for MR performance prediction depend on the solubility profile of the drug as well as the formulation type. In cases where the drug has good solubility and is formulated in a robust formulation, simpler dissolution methods may be adequate to reflect the *in vivo* dissolution behaviour. For instance, it was shown for the BCS class I compound zolpidem that the dissolution rate obtained using the USP apparatus II (paddle apparatus) QC method could appropriately describe the pharmacokinetics in conjunction with PBPK modelling under fasting conditions [26]. On the other hand, in the case of the poorly soluble compound nifedipine, dissolution results with the QC method based on USP apparatus II were misleading, and a much better representation of the dissolution behaviour *in vivo* was achieved with the more complex methods using USP apparatus III, especially for administration in the fed state [27].

Within OrBiTo, protocols were developed and validated to simulate release over the GI tract in both fasted and fed state conditions for both USP apparatus III and USP apparatus IV. Methods were optimised in a systematic approach using drugs from various BCS classes (mesalamine, zolpidem, nifedipine, ciprofloxacin) formulated in dosage forms with different formulation principles and food effect behaviour [25–27]. Comparing the USP apparatus III to the USP apparatus IV under the applied experimental conditions, it appears that when working on moderate to highly soluble compounds, the USP apparatus III is preferable due to the excessive consumption of dissolution media in the flow-through cell for formulations with a long duration of release. Also, in situations where the intensity of agitation may be important for the specific drug product, USP apparatus III may be preferable over USP apparatus IV, as demonstrated with the matrix-type dosage form of nifedipine [27]. However, the USP apparatus IV may serve as the preferred option for fine multi-particulate formulations if there is a risk of losing drug particles through the USP cylinder mesh during the experiment (*i.e.* for particles < 420 µm) and may also be beneficial for drugs with very poor solubility, as it is easier to maintain sink conditions due to the higher media volumes that may be used [27].

Ring studies across consortium partners were performed to evaluate inter-laboratory variability and the robustness of the proposed methods with USP apparatus III and IV. The results will be presented in a forthcoming paper.

3.2. Predicting dissolution in the lower intestine

Dissolution in the lower intestine, *i.e.,* the distal ileum and proximal colon, is of interest when drugs are administered orally in dosage forms with extended and/or delayed absorption kinetics. Relevant scenarios include IR products of high dose drugs and modified release products for which dissolution is driven primarily by physicochemical factors (*e.g.* pH). It was shown that *in vitro* two-stage dissolution testing under conditions simulating the environment in the lower intestine are useful for evaluating the impact of formulation or dosing conditions on the average plasma profile in adults [9,10] (Fig. 3).

3.3. Evaluating the impact of GI transfer in the fasted state on drug product performance

To mitigate the solubility limitations of many new drug candidates, one increasingly common approach has been to produce supersaturated concentrations of drug in the GI lumen, since more drug in solution means that more is available for absorption. However, the

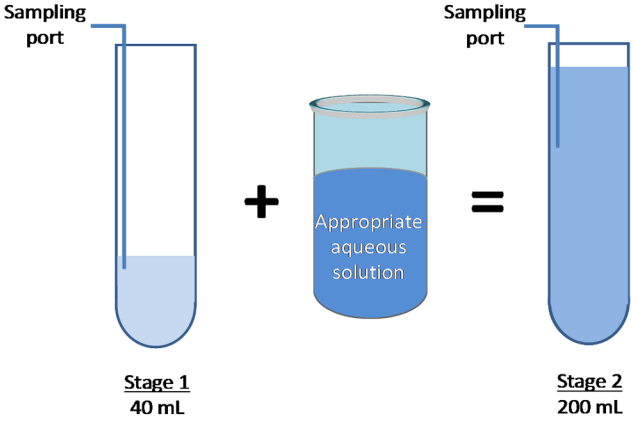


Fig. 3. Schematic representation of the two-stage single-compartment model for evaluating dissolution in distal ileum and proximal colon using a Distek® mini-vessel (ca. 200 mL) with either Level I or II biorelevant media and the Distek® mini-paddle, or Level III biorelevant media and an in-house mini-paddle system. Reprinted with permission from [1]. Copyright (2017) American Chemical Society.

supersaturated state is thermodynamically unstable and therefore has a propensity to precipitate. A range of formulation approaches is available to achieve supersaturation and, with the inclusion of various excipients, drug precipitation in the GI lumen can often be inhibited [33–35].

Even when not deliberately applying a formulation approach to achieve supersaturation, the transfer from the acidic stomach to the approximately neutral small intestine can also induce supersaturation for weak bases. Again, precipitation may occur as a consequence of the thermodynamically unstable supersaturated state [36].

A plethora of factors can influence the supersaturation and precipitation behaviour of these drugs and formulations. These factors are related directly to the properties of the drug itself (including ionization characteristics, solubility and solubilisation by bile acid micelles and permeability), the formulation (nature of the formulation and excipients used) and the environment in the proximal GI tract (such as pH, gastric emptying rate, volumes and composition of lumenal fluids). Consequently, all these parameters need to be considered when evaluating the supersaturation and precipitation behaviour for predicting *in vivo* behaviour.

3.3.1. Optimised use of simple transfer methods

As part of the OrBiTo project, the original transfer model first described in 2004 [36], was optimised to make it more physiologically relevant and thus improve its ability to reliably predict *in vivo* behaviour. The transfer model comprises a two-compartment setup simulating the stomach (donor) and proximal intestinal (acceptor) compartment utilizing a USP II dissolution apparatus. Through the transfer of the gastric media (containing the disintegrating formulation/dissolving drug) from the stomach and into the intestinal compartment, the gastric emptying process can be simulated. The model was optimized with respect to gastric emptying patterns, volumes and media composition to better reflect the proximal GI tract under fasting conditions [37]. Characterization of the degree of supersaturation and the precipitation kinetics enabled qualitative prediction of the *in vivo* behaviour of the different formulations [37–39]. For example, the *in vitro* evaluation of a capsule and solution formulation of itraconazole (Sporanox®), the relative *in vitro* behaviour of the solution versus capsule formulation reproduced the pharmacokinetic behaviour of the two formulations *in vivo*.

As the transfer model can potentially overpredict the precipitation behaviour for high permeability drugs due to the lack of an absorption component in the *in vitro* setup, physiologically based pharmacokinetic modelling (PBPK) was implemented to assess the evaluation of the significance of the supersaturation and precipitation observed *in vitro* for ketoconazole on its *in vivo* absorption characteristics [37,38]. For the highly permeable ketoconazole, coupling the *in vitro* data to a PBPK model revealed that precipitation would not occur *in vivo,* since the critical concentration for precipitation was not achieved in the intestinal compartment. These simulations were in accordance with the lack of precipitation observed in the intestinal lumen in the *in vivo* studies [40], and furthermore the plasma concentrations were accurately predicted with the transfer model/PBPK approach.

3.3.2. Artificial stomach duodenum model

The artificial stomach duodenum (ASD) model is a transfer model based on two chambers, one representing the stomach and a second representing the duodenum. It was originally developed as an *in vitro* alternative to studies with duodenally fistulated dogs, which had demonstrated that the amount of drug in solution in the dog duodenum was an excellent surrogate for predicting relative bioavailability of different formulations [41].

Using data from *in vivo* studies conducted within the OrBiTo project [22,42,43], the ASD operating parameters were optimized in terms of gastric and duodenal volumes and the implementation of a more physiologically relevant gastric transfer rate. Studies have been conducted with ketoconazole and posaconazole to assess the impact of the changes on the prediction of supersaturation and precipitation for these weak bases. Since OrBiTo *in vivo* studies had demonstrated that 10 min was more representative of the gastric emptying in the fasted state [22], implementing a shorter gastric emptying half-life (10 min) was compared to a gastric emptying half-life of 20 min for ketoconazole. However, it was found that the dose administered (100 mg and 300 mg) had a greater impact on supersaturation and precipitation than the gastric emptying rates. With posaconazole, the *in vitro* profiles measured in the duodenal ASD compartment for solution and suspensions demonstrated a similar maximum precipitated fraction to previously reported *in vivo* profiles in the upper small intestine of healthy adults [12].

3.3.3. The BioGIT model

The Biorelevant Gastrointestinal Transfer (BioGIT) model is an open *in vitro* set-up for simulating the continuous GI transfer process *in vivo* and estimating the apparent drug concentrations and solid fraction in upper small intestine after co-administration of a dosage form with a glass of water to fasted adults [44]. It consists of three compartments, gastric, duodenal, and a reservoir compartment and it is implemented by using commercially available equipment (Fig. 4). Conditions in the duodenal compartment account for both the transport of a highly permeable API across the epithelium and the transit through the upper small intestine. For solution formulations, the fraction precipitated in the upper small intestine is estimated. Part of each sample from the duodenal compartment can be used for measuring apparent equilibrium solubility and, therefore, apparent supersaturation in upper small intestine can also be estimated. The BioGIT model was shown to be useful for evaluating formulation performance in the upper small intestine, as well as the impact of dose and formulation on concentrations in the upper small intestine and on early exposure, after administration of conventional or enabling products of highly permeable drugs [13,16,44,45] (Fig. 4). Also, it has recently been shown to be useful for informing physiologically based pharmacokinetic modelling approaches [46].

3.4. Accounting for absorption in formulation evaluation

Traditionally, *in vitro* formulation evaluation has been based mainly on dissolution testing, largely ignoring the epithelial permeation of drugs that occurs *in vivo*. For highly permeable but poorly soluble BCS class II drugs, however, the kinetics of dissolution and, in case of supersaturated concentrations, precipitation may be reduced by the rapid removal of drug via permeation. To account for this effect, it may be valuable to include an absorption compartment in dissolution setups. Another benefit of implementing permeation into dissolution testing is that, assuming that only the free form is available for absorption, the performance of a dosage form can be assessed by looking at the drug fraction accessible for absorption (*i.e.* bio-accessible), which is not always equal to the apparently dissolved fraction [47].

3.4.1. Accounting for the effect of absorption on dissolution and precipitation kinetics

The principle of biphasic dissolution using a water-immiscible organic solvent to simulate an absorptive compartment has been used since 1967 and was first described by Niebergall et al. [48]. In a ring study across OrBiTo partners, various small-scale biphasic dissolution systems were applied. The systems from Merck, Sirius/Pion (both using inForm©) [49] and Boehringer-Ingelheim [50] were compared and solid dispersions of a poorly soluble development compound from Janssen Pharmaceutica were tested. The experimental setup at the three laboratories differed in the following aspects:

- volume and type of aqueous phase (phosphate buffer or biorelevant media)
- volume and type of organic phase (octanol, nonanol or decanol)

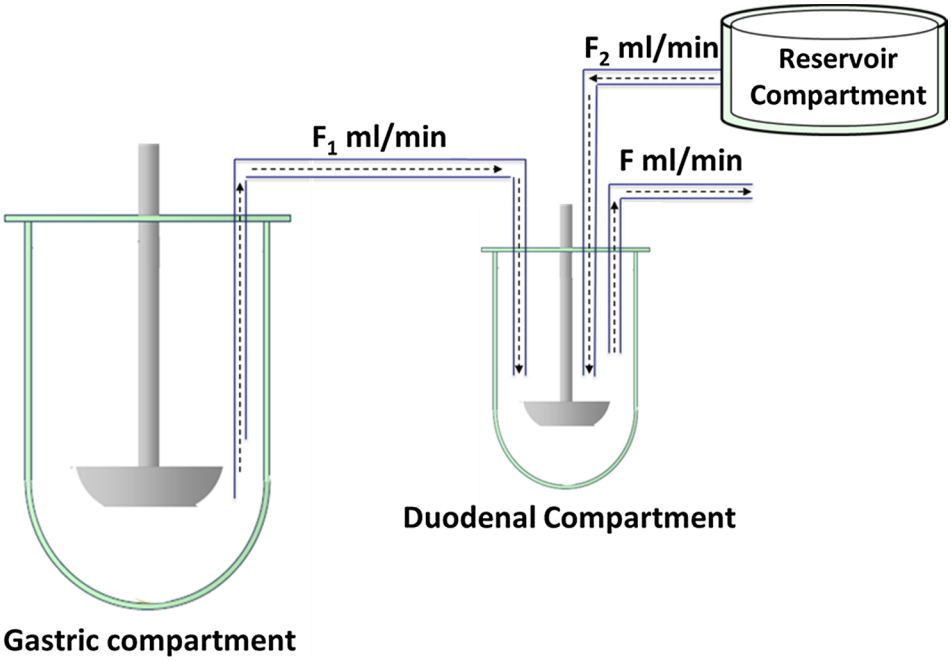


Fig. 4. Schematic representation of the BioGIT model. F_1 and F_2 are the incoming flow rates and F is the outgoing flow rate; $F = F_1 + F_2$.

- addition of samples (solution or powder)
- geometrics of the vessel and stirrer (flat or USP II apparatus-like; paddle or stirring bar)
- quantification method.

Even though the setup was specific for each laboratory, the results revealed the same qualitative trends and it was shown in all cases that the addition of the organic layer as a sink decreases the degree and rate of precipitation. The way how the test sample is added, type of aqueous media, and doses to be tested were identified as critical factors which need to be aligned in future studies. A best practice protocol for performing of biphasic experiments is currently being developed and will be applied in a follow-up ring study with fenofibrate solid dispersions, in which the *in vivo* relevance will also be evaluated.

3.4.2. Optimized/new tools to assess permeation from complex luminal media

Intestinal drug permeation from luminal media can be assessed *in vitro* by cell-based systems such as the human colorectal adenocarcinoma (Caco-2) and the Madin Darby canine kidney (MDCK) cell line [51]. In OrBiTo, one such experiment investigated the permeation potential of itraconazole in the presence of hydroxypropyl-β-cyclodextrin: following intake of the oral solution Sporanox®, aliquots of human intestinal fluids were applied to the apical side of a Caco-2 cell insert system immediately upon aspiration [52]. The observed *in vivo* solubility-permeability interplay for the cyclodextrin-based solution of itraconazole could be captured when assessing permeation from *in vitro* dissolution samples across a Caco-2 cell monolayer [20]. In addition, Forner et al. used a Caco-2 cell monolayer and rat intestinal sheets in a dissolution/permeation set-up to predict the *in vivo* absorption of clarithromycin from four different formulations, differing in particle size and excipient concentration. The enhancing effect of reduced particle size and adding surfactants on the *in vivo* absorption was best depicted by the dissolution/permeation experiments across Caco-2 cells [53]. The authors suggested that the presence of an enlarged mucus layer *ex vivo* may hamper the flux across the rat intestinal sheets.

As lengthy preparation steps along with monolayer integrity issues limit the attractiveness of cell-based systems for use in drug development, cell-free permeation systems are gaining interest. As such, the artificial membrane insert (AMI) system comprising of a simple regenerated cellulose membrane, mounted between two plastic rings, was developed within the OrBiTo project [54]. By applying a sufficiently high stirring speed together with a surfactant-based solution in the acceptor compartment, a reasonable correlation was demonstrated with the well-established Caco-2 system for 14 different poorly-water soluble drugs. Following the validation study of this flexible permeation system, the usefulness of the AMI-system to assess permeation from complex luminal media, and in particular, as a predictive tool for the early-stage evaluation of absorption-enabling formulations, was explored [20]. In combination with a simple dissolution test (as illustrated in Fig. 5), the AMI-system was able to capture the impact of (i) formulation pH on posaconazole absorption, (ii) dilution on cyclodextrin-based itraconazole absorption, and (iii) food intake on fenofibrate absorption. These data indicate that the AMI-system can be beneficial over cell- and tissue-based systems to implement permeation in early-stage formulation evaluation since it easily resists the detrimental effects of food components/pharmaceutical excipients.

A similar artificial membrane was used in a different set-up to investigate the impact of food on fenofibrate absorption [19]. To set up a predictive *in vitro* model that could estimate the bioaccessible fraction of fenofibrate, the implementation of a permeation bag, made of a regenerated cellulose membrane, in dissolution testing appeared to be essential. The setup of this model is depicted in Fig. 6. Besides the implementation of a permeation bag, selection of appropriate biorelevant media was critical to adequately simulate the food-induced solubility-permeability interplay, as observed *in vivo*.

3.5. Mimicking the impact of gut motility

3.5.1. New insights into disintegration as a tool to assess the impact of GI motility

Disintegration testing has become an important test in the pharmaceutical industry, and disintegration test procedures for various dosage forms have been described in the various pharmacopoeia. Since even complete disintegration does not necessarily imply complete dissolution, more research focus has been devoted to dissolution rather than disintegration testing. However, in some cases, a disintegration test may act as a surrogate for dissolution testing, and as reviewed in Al-

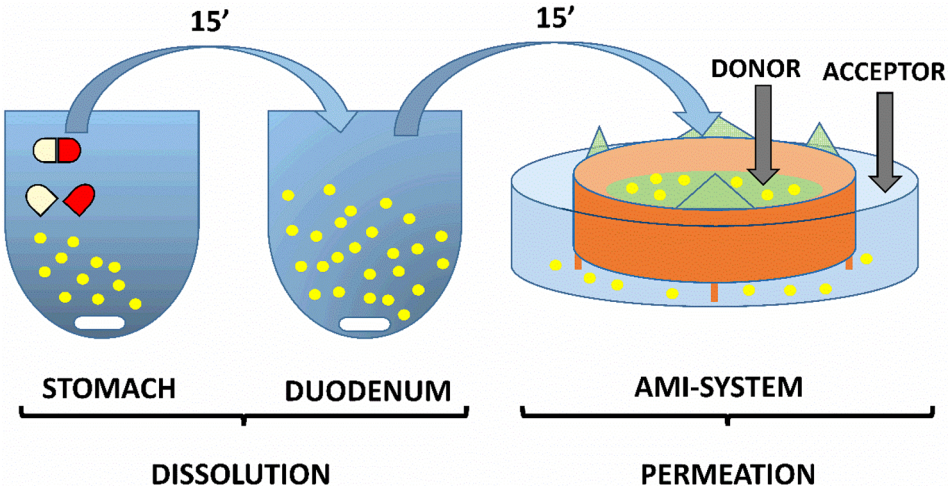


Fig. 5. Schematic overview of the combined dissolution-permeation set-up using the AMI-system as barrier simulating the intestinal epithelium.

Gousous and Langguth, the disintegration test can be used as a surrogate for dissolution testing if certain conditions related to the type of solid dosage form, the dose to solubility ratio of the drug, and the rate of dissolution are met [55]. Other challenges also need to be addressed for the proper use of disintegration testing as a dissolution test surrogate. For example, the specifications generally applied in disintegration tester are not sufficiently narrow to exclude test results which are significantly different from each other. Moreover, the biorelevance of the hydrodynamics and the current media used for *in vitro* disintegration testing, in addition to the mechanical stresses involved, are questionable. In particular, the influence of viscosity on the disintegration behaviour of solid dosage forms, which has been largely ignored in the past, was extensively investigated within the OrBiTo project [56–58] and formulation strategies aiming at robust *in vivo* disintegration have been introduced [59].

Within the OrBiTo project, the hydrodynamics in the Ph.Eur./USP disintegration tester were characterized using computational fluid dynamics (CFD) combined with particle image velocimetry [60]. CFD simulations were performed with different Newtonian and non-Newtonian fluids representing both the fasted and fed states and results indicated that the current design and operating conditions of the compendial disintegration test device are not representative of the *in vivo* situation. The forces acting on the dosage form are less aggressive compared to the peak forces *in vivo* and the lack of peristaltic contractions, which generate hydrodynamics and shear stress *in vivo*, is a further disadvantage of the current compendial device. In response to these deficiencies, a modified *in vitro* disintegration test device was designed to make the hydrodynamics more biorelevant [61]. By application of a computerized numerical control, a variety of physiologically relevant moving velocities and profiles can be applied. CFD characterized the hydrodynamic and mechanical forces present in the probe chamber for a variety of device moving speeds. Furthermore, it was shown that the disintegration times of IR tablets increased with decreasing moving velocity and this was predicted by CFD simulations quantifying the shear stress on the tablet surface. The modified disintegration test device is thus a potentially valuable tool for biorelevant *in vitro* disintegration testing of solid oral dosage forms.

3.5.2. *New/optimised tools to assess the impact of GI motility in dissolution testing*

GI motility can have dramatic effects on the drug release behaviour of solid oral dosage forms as demonstrated for various ER formulations by Garbacz and Weitschies using the Dissolution StressTest device [62]. Within OrBiTo, biorelevant *in vitro* dissolution tools able to simulate human peristalsis were further optimised based on recent *in vivo* data gathered with telemetric motility capsules [4,5]. Schneider and coworkers illustrated that these *in vivo* data can be directly implemented into biorelevant *in vitro* tools, whereas the intermittent intensity of physiological mechanical stresses cannot be simulated by use of compendial dissolution test devices such as USP apparatus II or III [6]. The optimised *in vitro* tools were applied to testing of IR as well as ER formulations [63–65]. In the first study, the Dissolution StressTest device was used to characterize drug release from different mesalazine formulations. These experiments revealed that some of the tested

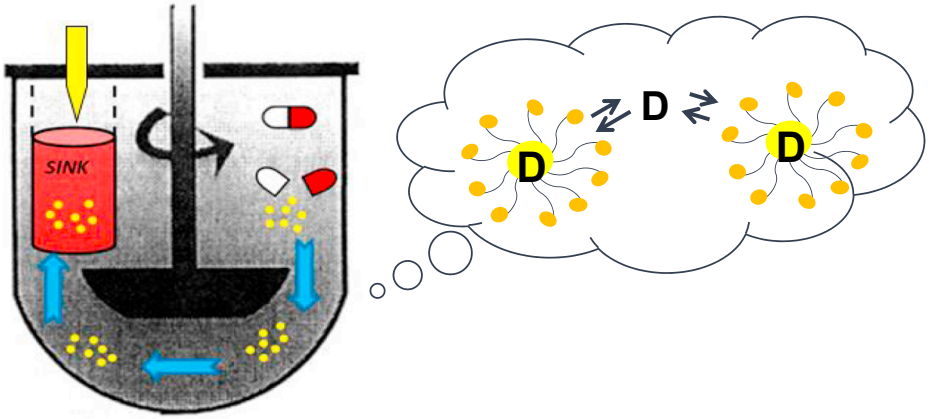


Fig. 6. Representative illustration of the combined dissolution/permeation setup using a permeation bag to simulate intestinal absorption.

formulations experienced dose dumping-like drug release behaviour after simulation of pressure events of high intensity [63]. In another study, the Dynamic Open Flow-Through Test Apparatus (DOFTA), a biorelevant dissolution device dedicated to the simulation of the fasted stomach, was applied to study drug release under realistic conditions from two different formulations of N-acetylcysteine (tablet vs. granule formulation). In these experiments, the impact of the interplay between gastric emptying and peristalsis on the onset of drug plasma concentrations after drug administration in fasted state was explored [64]. Another tool used to simulate GI motility is the TIM-agc (agc = advanced gastric compartment) developed by TNO in the Netherlands. With the aid of the SmartPill®, it was shown that physiologically relevant pressures can be simulated in the TIM-agc [66].

3.6. Accounting for food and digestion processes

3.6.1. New insights into methods for assessing the fed state

The intake of food leads to various changes with respect to the physiological conditions present in the upper GI tract. These include different physicochemical, enzymatic and mechanical parameters in the stomach and small intestine, all of which are highly relevant for oral drug delivery. Using the insight of the fed state conditions gained in recent *in vivo* studies, in which the techniques described in Section 2 were applied, the existing *in vitro* tools were optimized within the OrBiTo project. Two different approaches were followed.

The first approach was to simulate variation in individual parameters (*e.g.,* pH value, gastric emptying, peristalsis) of gastrointestinal transit. A biorelevant dissolution test system that can be applied for such purposes is the Fed Stomach Model (FSM) [67]. This system was developed to simulate mechanical aspects of the fed stomach such as dosage form movement or pressure events occurring in the stomach. In addition, it also considers the localisation behaviour of oral dosage forms, as this has been shown to be extremely important [68]. Whereas in proximal parts of the stomach, an oral dosage form is exposed only to small stresses, in distal parts high mechanical stresses can arise and thus, promote drug release. In addition, the FSM can also be used to change the luminal parameters (*e.g.,* pH) with time in the gastric compartment and to simulate different rates of gastric emptying. Other groups used the USP apparatus III (*i.e.,* reciprocating cylinder) to investigate food effects. For instance, Andreas and co-workers successfully forecasted the formulation-dependent food effect of nifedipine modified-release tablets [27].

The second approach was based on a complex simulation of GI physiology in the fed state in order to study drug release under physiologically relevant conditions. For this purpose, *in vitro* models such as the Dynamic Gastric Model (DGM) or the TNO TIM-1 system (see Section 3.7) were used. These tools are able to simulate physiological processes such as gastrointestinal secretion, digestion of nutrients, motility and luminal transfer of contents in a realistic manner and therefore, they can be helpful to predict the presence and direction of food effects [69]. For instance, Verwei and co-workers showed how the TIM-1 system was used to investigate the presence of food effects in case of several poorly water-soluble drugs [70].

3.6.2. New insights into gastric and intestinal lipolysis for lipid-based formulations

When evaluating the performance of lipid-based drug delivery systems (LbDDS), the impact of lipolysis typically needs to be considered, since most lipids will be digested by lipases present in the GI tract. Traditionally, *in vitro* lipolysis models only simulate lipolysis in the small intestine, which is primarily catalysed by the pancreatic triglyceride lipase (PTA) [71]. However, this approach ignores the fact that about 10–30% of the triglyceride lipolysis is catalysed by gastric lipase, which is secreted by the gastric mucosa in the human, and that gastric lipase can remain active in the small intestine [72]. In addition, studies in rats, carried out as a part of the OrBITo project, showed that drug precipitation from LbDDS can occur *in vivo* in the stomach of rats. Therefore, it is of interest to develop an *in vitro* model that also simulates the lipolysis in the stomach. Microbial lipases are often applied to simulate gastric lipolysis despite their potential shortcomings with regard to activity and specificity, since human gastric lipase is not commercially available. In order to clarify the situation, three lipases were compared: a microbial lipase (*Rhizopus oryzae*; ROL), rabbit gastric lipase (RGL) and recombinant human gastric lipase (rHGL), using infant formula milk as substrate [73]. The study revealed that the three lipases differed with regard to the extent of digestion, affinity to different fatty acids and morphology of the oil droplets during *in vitro* gastric digestion. Human gastric lipase has a higher affinity towards medium chain than long chain fatty acids. Compared to existing *in vivo* data [74], ROL had a much higher extent of digestion and an equal affinity to long and medium chain fatty acids [74]. By contrast, the extent of digestion by RGL and rHGL was within the expected range and displayed a preference for medium chain fatty acids. However, of the two, only rHGL resulted in release of long chain fatty acids [73,74]. Thus, when gastric digestion of lipid substrates with different chain lengths are being studied, it is important to consider which gastric lipase is used.

3.6.3. Predictive ability of two-step digestion models

As a part of the OrBiTo project, a two-step, small scale *in vitro* lipolysis model, simulating both digestion in the stomach and the small intestine, was established [75]. The two-step *in vitro* lipolysis model is depicted in Fig. 7. The model was tested using two LbDDS from Sanofi,

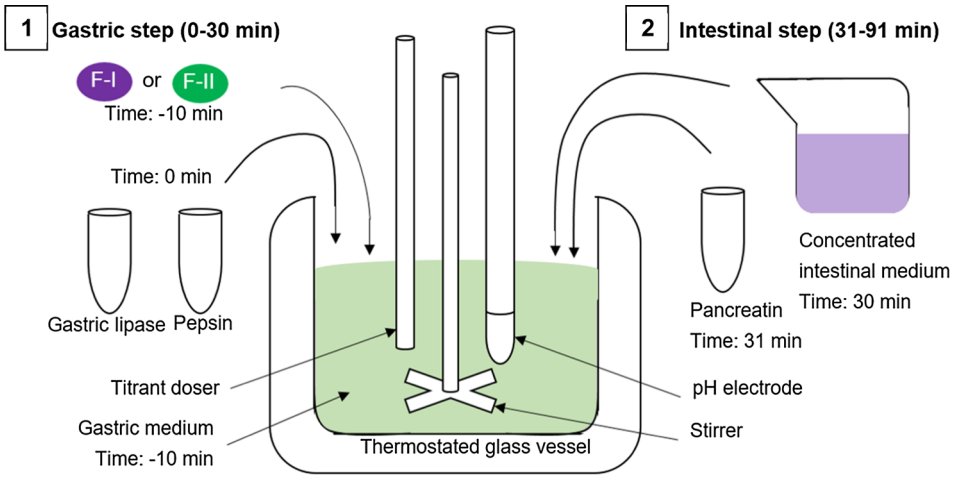


Fig. 7. Representative illustration of the two-step (*i.e.,* from stomach to intestine) *in vitro* lipolysis model.

for which data from an oral pharmacokinetic study in beagle dogs were available. Drug solubilization was compared using the two-step, small scale *in vitro* lipolysis model and a traditional intestinal *in vitro* lipolysis model. Compared to the *in vivo* study of the two LbDDS in beagles, the T_{max} and C_{max} of each LbDDS was better reflected by the solubilization profile of the two-step, small scale *in vitro* lipolysis model than the one-step intestinal *in vitro* lipolysis model [71,76]. Further, the two-step, small scale *in vitro* lipolysis model provided an improved understanding of the *in vivo* behaviour of the tested LbDDS. It is therefore recommended to take gastric *in vitro* digestion into account when developing LbDDS. However, it has to be noted that this very simple model does not consider gastric emptying rates, a feature that has been included in some recent *in vitro* lipolysis models [77].

3.7. Dynamic multi-compartmental "GI tract in the lab" systems

3.7.1. Dynamic gastric model/model gut

Complex *in vitro* models of the upper GI tract attempting to mimic the dynamic GI environment in terms of hydrodynamics and media characteristics are potentially of value for the development and understanding of new oral pharmaceutical products [78]. The Dynamic Gastric Model (DGM) is one such model. It can simulate the pH and emptying profile of the fasted stomach and can also accommodate and digest a meal, *e.g.,* the FDA breakfast. It can thus evaluate the *in vivo* behaviour of a dosage form in both the fasted and fed state. The DGM can be combined with an intestinal model to simulate the fate of the dosage in the duodenum and small intestine after transfer from the stomach. The ability of the DGM to predict the *in vivo* performance of dosage forms containing posaconazole (Noxafil®, 40 mg/ml) and diclofenac (Cataflam®, 50 mg), were tested during the OrBiTo project.

Posaconazole was dosed to the fasted state DGM in the Noxafil® formulation adjusted to pH 1.6 and 7.1 respectively [79], in the same manner as described in a clinical study by Hens et al. 2016 [12]. When dosing posaconazole at pH 1.6, nearly all drug was in solution in the DGM. Upon transfer to the duodenal compartment and the concomitant pH jump to 6.5, precipitation was observed. However, supersaturation was seen for almost an hour, in line with the *in vivo* observations. When administering at pH 7.1 in the gastric compartment, barely any drug was initially in solution in the DGM. As the pH dropped and the DGM emptied, posaconazole dissolved and towards the end of the gastric phase, all drug remaining in the gastric compartment was in solution. In the duodenal compartment, posaconazole was mainly found in precipitated form, with no supersaturation occurring. These events were also in line with the *in vivo* situation.

Diclofenac was dosed to the DGM in the fasted and fed state [80], as described in a clinical study [17]. The DGM was set to operate in its standard fasted and fed state modes, and the samples emptied from the DGM were incubated under duodenal conditions, by addition of bile salts and pancreatin. The fasted samples ejected from the DGM showed a high degree of solid drug present, while the fed state samples contained more drug in solution, in line with the observations *in vivo*. In the duodenal step, the drug rather quickly went in solution, also similar to the *in vivo* observations.

Overall the two studies showed that the DGM may be useful for assessing the physical behaviour of drugs and dosage forms during residence in the stomach, an important aspect of understanding the *in vivo* behaviour of oral dosage forms.

3.7.1.1. Optimisation of the TNO intestinal model (TIM-1) and tiny-TIM model. The TIM-1 system aims to simulate the physiological luminal conditions of the GI tract as closely as possible. Given the multitude of GI parameters that are simulated, this dynamic system is relatively complex. Therefore, a simplified dynamic two-compartmental tiny-TIM system was developed, consisting of a gastric compartment and one, rather than three compartments for the small intestine. In addition, the conventional gastric compartment was optimized to the TIM advanced gastric compartment (TIMagc), which better reflects the realistic shape and dynamic gastric conditions observed in the human stomach [81].

Alongside these hardware changes, computer protocols and buffers have been optimized. The new settings and conditions were subsequently tested in a comparative OrBiTo ring study in TIM-1 systems, which indicated that the acetaminophen bioaccessibility under fasted state and fed state varied minimally among the different TIM-1 users. This indicates that, although the holistic simulation of multiple parameters leads to a complex experimental system, accurate automation and control of the process allows reproducible experiments with little inter-laboratory variation.

3.7.1.2. Predictive ability of the TNO intestinal model (TIM) and tiny-TIM model. The performances of TIM-1 and tiny-TIM were investigated by comparing the bioaccessibility data for four different low soluble drugs (ciprofloxacin, posaconazole, nifedipine and fenofibrate) in different dosage forms under fasted and fed state conditions [70]. The results from the two systems were comparable: both systems correctly predicted the presence (posaconazole) or absence (ciprofloxacin) of a food effect on bioaccessibility and the higher bioaccessibility of fenofibrate from the nanoparticle formulation compared to the microparticle formulation. TIM-1 provided a more detailed simulation of bioaccessibility from different regions of the small intestine and could determine the non-bioaccessible fraction by the presence of an ileum effluent. However, tiny-TIM, with direct filtration from a single small intestinal compartment directly below the gastric compartment, predicted bioaccessibility of compounds with rapid absorption better. The advanced gastric compartment (TIMagc) was used to replicate a clinical study by investigating the disposition of a diclofenac tablet (Cataflam®) under fasted state conditions and fed state conditions using a liquid Ensure Plus® meal [17]. Gastric luminal sampling indicated substantial amounts of solid diclofenac in the stomach under fasted, as well as fed state conditions, which then dissolved rapidly upon entry in the small intestine, in line with *in vivo* observations. The system could identify individual factors influencing tablet disintegration rate, namely gastric pH and meal composition, indicating an effect of pepsin activity on tablet disintegration under fed state conditions.

4. Methodologies for selecting the appropriate *in vitro* tool based on IMI OrBiTo studies

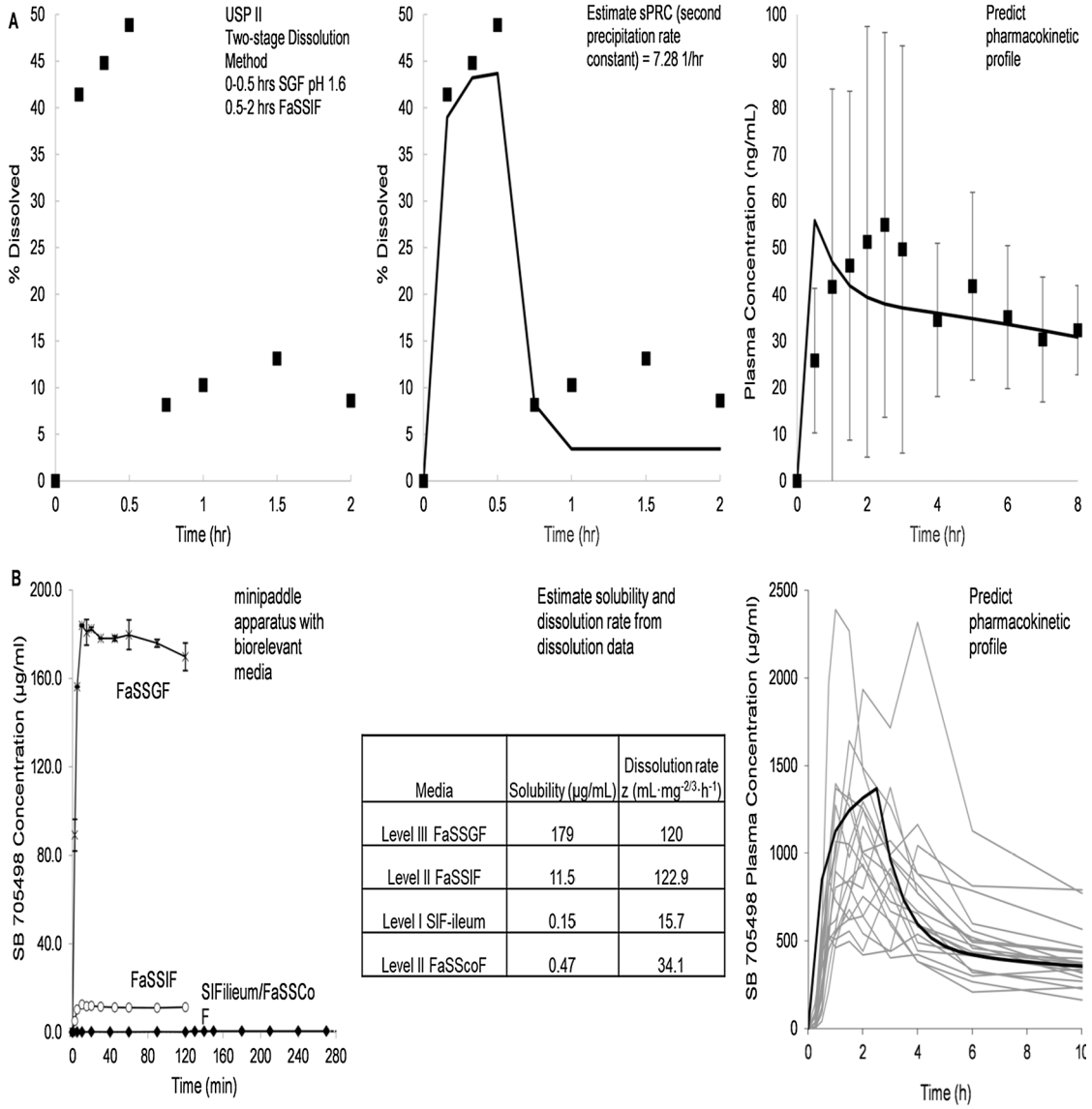
As documented in the preceding sections, work package 2 of the OrBiTo project covered a variety of different *in vitro* tools for the investigation of immediate and modified release formulations ranging from compendial-based methods (USP apparatuses II-IV) to systems with a more physiological representation of the hydrodynamic forces such as the Dissolution Stress Test or the TIM models. With the availability of multiple possibilities to perform dissolution tests, it was a key task of the OrBiTo project to guide scientists in selecting the appropriate *in vitro* tool. For that reason, a web-based decision tree was designed, providing high-level overviews of the various methods [2]. Depending on the drug/formulation combination and the desired level of complexity, short summaries of the proposed dissolution methods are provided, including a brief description of the methodology, exemplary experimental conditions as well as literature references for further information. The decision tree can be found under www.orbito-dissolution.eu. Currently, the decision tree addresses only the *in vitro* tools developed or optimized within OrBiTo. However, it is anticipated that the decision tree will be continually extended and updated in the future, guided by an independent consortium committee.

In a complementary project within OrBiTo, the Development Classification Scheme (DCS), first published by Butler and Dressman in 2010 [82], was updated to align the analysis even more closely to the needs of formulation development [83], creating the refined DCS (rDCS). For example, since the dose of the drug has often not been pinpointed at the time when formulation development is started, a

range of potential doses can be applied in the rDCS. Further, tests are stratified into standard and customized assessments, depending on the complexity of the drug's physicochemical properties. Last but not least, the rDCS enables benchmarking of solubility and permeability studies conducted according to in-house protocols, giving companies more flexibility in their protocol design. In a subsequent article, case examples illustrating the application of the rDCS will be presented.

5. Integration of *in vitro* models with PBPK modelling – An update

Traditionally, in a pharmaceutical development setting, the biorelevant media and *in vitro* models are applied primarily at an early stage of drug product development to discriminate the rank order of drug formulations in terms of anticipated *in vivo* performance. Recently, there has been an increased interest towards quantitative use of these data for clinical performance predictions (*i.e.* prediction of plasma concentration profiles). Whereas predictions using QC dissolution data are typically pursued via the traditional IVIVC approach, dissolution data acquired with biorelevant methodologies are often incorporated into PBPK models. A typical workflow is presented in Fig. 8, according to which dissolution data are first modelled to obtain relevant parameters that are then applied in the PBPK model to predict the drug plasma concentrations. For example, Ruff et al. and Pathak *et al.* utilized data from a transfer method to estimate precipitation kinetics that proved useful for successful prediction of plasma concentration profiles [37,38]. Focusing again on precipitation kinetics, Hens et al. utilized data from the somewhat simpler, but widely used, two-stage dissolution setup to model both the intraluminal concentrations and the plasma levels for posaconazole suspension [84] (Fig. 8A). Georgaka et al. utilized dissolution data in biorelevant media that simulated different regions of the GI tract to simulate absorption of a high dose for low solubility compounds (SB705498 shown as an example in Fig. 8B with a table describing the estimated solubility and dissolution rates derived from *in vitro* experiments) [10]. Furthermore, Andreas et al. utilized dissolution data from modified release formulations in both prandial states to simulate food effects [26]. With the increased emphasis by regulatory agencies on increased product/biopharmaceutics understanding and, subsequently, clinically relevant specifications, we expect to see further efforts to increase utilization of biorelevant dissolution data in PBPK modelling, not only to help development decisions but also to inform regulatory CMC arguments. Modelling of the dissolution data in biorelevant dissolution methods can also help with the



Media	Solubility (µg/mL)	Dissolution rate z (mL·mg$^{-2/3}$·h^{-1})
Level III FaSSGF	179	120
Level II FaSSIF	11.5	122.9
Level I SIF-ileum	0.15	15.7
Level II FaSScoF	0.47	34.1

Fig. 8. Workflow for incorporation of dissolution data in PBPK modelling. (A) Incorporation of precipitation information from a two-stage dissolution setup. Data based on Hens et al. [84] (B) Incorporation of dissolution data across biorelevant media to map dissolution across the entire GI tract. Data from Georgaka et al. [10].

understanding of rate-determining steps in *in vivo* dissolution and can serve as a precursor to the development of QC dissolution methods, as recently suggested as part of a clinically relevant dissolution specifications roadmap [85].

6. *In vitro* models for predicting drug absorption: The future

As a result of the OrBiTo collaboration, we have developed and optimized a wide range of *in vitro* models for the prediction of oral dosage form behaviour, and mapped out through a decision tree how they could be optimally applied. These models range from the relatively simple (tests typically based on compendial apparatus) to quite complex, multi-compartmental tools. Some of these more complex tools are designed to tackle a specific behaviour observed *in vivo* (*e.g.,* the impact of gastric motility, or intestinal precipitation), whilst other complex tools such as the TIM-1 model are designed to mimic multiple aspects of *in vivo* behaviour in a single test. Some of these tools, especially those with greater complexity, are anticipated to find application as "stand-alone" holistic tools to predict formulation performance. Other, simpler tools will increasingly be used as improved, biorelevant inputs into PBPK models. With the availability of a wider and more robust *in vitro* toolset, we anticipate the following changes for future benefit of oral product development:

(1) Confidence to select the right *in vitro* tool(s) to understand the behaviour of each specific oral formulation in the GI tract.
(2) Replacement of pre-clinical animal experiments for formulation selection/optimization during early product development with appropriate *in vitro* tools.
(3) Widespread use of biorelevant *in vitro* tools to identify likely critical quality attributes (CQAs) of new oral products, with the data generated then being used to inform QC dissolution method selection and eventually specification setting.
(4) Fewer pharmacokinetic "surprises" in relative bioavailability studies, and a reduced frequency of failed formal bioequivalence studies in humans.
(5) Increased use of biorelevant *in vitro* data in submissions to support regulatory filings.

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