Oral biopharmaceutics tools: Recent progress from partnership through the PEARRL collaboration

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Abstract

Objectives

To summarise key contributions of the PEARRL (Pharmaceutical Education and Research with Regulatory Links) project (2016-2020) to the optimisation of existing and the development of new biopharmaceutics tools for evaluating the *in vivo* performance of oral drug products during the development of new drugs and at the regulatory level.

Key Findings

Optimised biopharmaceutics tools: Based on new clinical data, the composition of biorelevant media for simulating the fed state conditions in the stomach was simplified. Strategies on how to incorporate biorelevant *in vitro* data of bio-enabling drug products into physiologically based pharmacokinetic modelling were proposed.

Novel *in vitro* biopharmaceutics tools: Small-scale two-stage biphasic dissolution and dissolution-permeation setups were developed to facilitate an understanding of the supersaturation effects and precipitation risks of orally administered drugs. A porcine fasted state simulated intestinal fluid was developed to improve predictions and interpretation of preclinical results using *in vitro* dissolution studies. Based on new clinical data, recommendations on the design of *in vitro* methodologies for evaluating the GI drug transfer process in the fed state were suggested.

Optimised design of *in vivo* studies for investigating food effects: A food effect study protocol in the pig model was established which successfully predicted the food dependent bioavailability of two model compounds. The effect of simulated infant fed state conditions in healthy adults on the oral absorption of model drugs was evaluated vs. the fasted state and the fed state conditions, as defined by regulatory agencies for adults. Using PBPK modelling, the extrapolated fasted and infant fed conditions data appeared to be more useful to describe early drug exposure in infants, while extrapolation of data collected under fed state conditions, as defined by regulators for adults, failed to capture *in vivo* infant drug absorption.

Conclusions

Substantial progress has been made in developing an advanced suite of biopharmaceutics tools for streamlining drug formulation screening and supporting regulatory applications. These advances in biopharmaceutics were achieved through networking opportunities and research collaborations provided under the H2020 funded PEARRL project.

Keywords

Biorelevant, *In vitro* testing, PBPK modelling, Preclinical, Pig Model, Paediatrics, Bio-enabling drug products, Food effect, Small-scale, PEARRL

1. Introduction

The increase in poorly water soluble drug candidates in the discovery pipelines has amplified the focus on improving oral biopharmaceutics tools to better understand drug product performance *in vivo*. Employing reliable and robust biopharmaceutics tools improves the efficiency and reduces timelines involved in the drug development process. Much progress has been made in recent years to improve the biopharmaceutics tools available to pharmaceutical scientists. These innovations include the introduction of biorelevant media[1–4] and the development of improved *in vitro* tools[5–8] in an effort to better replicate conditions of the gastrointestinal (GI) tract. Despite this progress, questions about the suitability of many biopharmaceutics tools remain unanswered.

PEARRL (Pharmaceutical Education and Research with Regulatory Links) is a recently completed EU funded project which brought together European Academic Institutions, Pharmaceutical industry, and Regulatory Agencies (www.pearrl.eu). A key goal of the project was to optimise existing and develop new biopharmaceutics tools to predict the *in vivo* performance of drug / drug products after oral administration. A few years ago, the OrBiTo consortium invited researchers to offer suggestions for improving and expanding decision trees for selecting the most appropriate *in vitro* methodology for release testing of solid oral dosage forms[9] and for selecting the most appropriate *in vivo* models for formulation development,[10] which may also be useful at the regulatory level.[11]

This article summarises the contribution of the PEARRL consortium to the optimisation of existing and the development of new biopharmaceutics tools for evaluating the *in vivo* performance of oral drug products during the development of new drugs and at the regulatory level.

2. Optimised biopharmaceutics tools

2.1 Biorelevant media reflecting the intragastric environment after a standard high-calorie high-fat meal

To assess the performance of oral drug products in the upper GI tract in the fed state, *in vitro* methods have, sometimes in combination with PBPK modelling techniques, been useful in reproducing mean plasma profiles and for improving the insight of the processes behind these food effects on the oral absorption of drugs.[12] Nevertheless, in most cases, the luminal environment has been simulated according to data previously collected after administration of a liquid meal with a comparable caloric content and, at times, of an analogous source to the standard high-calorie, high-fat meal (standard meal) used in the clinical studies in adults,[13,14] but not after administration of the standard meal itself.[12] Furthermore, the simulation of certain luminal characteristics in relevant methodologies, which may influence the performance of the drug product and the overall drug absorption process, such as the luminal viscosity and make-up of the micellar phase of contents in the upper intestinal lumen, has not been suitably justified.[15]

Based on information gathered 30 - 240 min after beginning the administration of the standard meal:[15]

- Level I simulation of gastric contents[4] can be attained by using one medium. Level I FeSSGF$_{late}$ (fed state simulated gastric fluid) proposed by Markopoulos *et al.* [4] adequately reflects the pH and buffer capacity of gastric contents after the standard meal; FeSSGF$_{late}$ should better be termed Level I FeSSGF-V2.
- A single Level II biorelevant medium for the simulation of the gastric environment in the fed state could also be considered. Level II FeSSGF$_{late}$ proposed by Markopoulos *et al.*[4] adequately reflects the osmolality and total molar concentration of lipid species in gastric contents after the standard meal and could be used for simulation of the intragastric environment. Level II FeSSGF$_{late}$ should be termed Level II FeSSGF-V2.
- Level II simulation of the aqueous phase of contents in the stomach or Level II simulation of the micellar phase of contents in the upper small intestine may need to be considered when investigating drug transport towards the GI mucosa.[16] Level II simulation of the aqueous phase of gastric contents in the fed state could be achieved by including 0.2 mM FAs and 0.5 mM PC in Level I FeSSGF-V2; the resulting medium should be termed Level II FeSSGF$_T$. Level II simulation of the micellar phase of contents in the upper small intestine could be achieved by including 6 mM bile acids, 0.2 mM MGs, 2 mM FAs and 0.9 mM lyso-PC in Level I FeSSIF-V2 and the resulting

medium should be called Level II FeSSIF$_T$. Nevertheless, until Level II FeSSIF$_T$ is shown to be sufficiently stable and suitable for use with cell cultures, FeSSIF-TM$_{Caco}$[17] could be employed.

- Level III biorelevant media should incorporate the higher luminal viscosity after the standard meal. Level III biorelevant media simulating fed gastric conditions should have a viscosity of approx. 800 mPa.s at 100 s^{-1}, while Level III biorelevant media simulating conditions in the upper intestinal lumen should be approx. 400 mPa.s at 100 s^{-1}.

2.2 In vitro characterisation of bio-enabling formulations and integration of the results into PBPK models

In recent years, high attrition rates have been observed in the pharmaceutical research and development sector due to the difficulties encountered in transforming emerging drug molecules into licensed medicines.[18] Most new drug candidates in todays' pipelines have suboptimal solubility and dissolution properties [19] and display poor bioavailability when administered orally. These new drug candidates require special formulation approaches to achieve acceptable oral bioavailability. Such novel formulations are usually referred to as "bio-enabling" formulations and are being employed ever more frequently during drug development.

Even with the intense focus on research relating to bio-enabling formulations, there are still many unanswered questions relating to their *in vivo* performance, potential changes in the physicochemical state of the drug substance, such as the formation of amorphous or nano-crystalline drug, interactions with polymers which may inhibit the precipitation of the drug substance, and a suitable method for incorporating this information into an *in silico* biopharmaceutics model. The complexity of the *in vitro* and *in vivo* dissolution processes have so far restricted the use of PBPK / absorption models to adequately simulate the *in vivo* performance of bio-enabling formulations.[20–22] A common issue to overcome when trying to characterise a bio-enabling formulation *in vitro* and build a suitable solubility/dissolution model is that properties of the pure unformulated drug substance might be different than the properties of the formulated drug product.

In particular, it is very important to identify and input a suitable "solubility" figure for the formulated drug in the following situations:

- when a "solubilisation ratio" to account for the *in vivo* solubilisation by the native surfactants in fasted and/ or fed state (e.g. as required by most commercially available *in silico* software) is to be estimated and

- when a simulation of the dissolution experiments (and eventually the *in vivo* performance of the drug formulation) is attempted, given that the rate of dissolution is proportional to the concentration gradient between the solubility at the particle surface in the media and the drug concentration in the bulk solution.

Dissolution experiments in Level II biorelevant media simulating the fasted and / or the fed state have proven more useful compared to the equilibrium solubility of the pure unformulated drug substance for identifying the "effective" solubility, modelling the dissolution experiments and forecasting the *in vivo* performance of bio-enabling formulations. In particular, two case example formulations were tested i.e., a nanosized[23] formulation of aprepitant and an amorphous solid dispersion (ASD)[24] of etravirine and the conclusions derived were the following:

- in the case where precipitation of drug is not expected in the GI lumen (deduced from the characteristics of the drug and/ or the results of the *in vitro* dissolution and transfer experiments), the maximum concentration of drug observed in the chamber during the dissolution experiment of the formulated drug in Level II biorelevant media can be used as a more appropriate ("effective") solubility of the formulated drug in the respective part of the GI lumen.
- in the case where precipitation is expected/ hypothesized, the concentration of drug observed after 24 h in the dissolution vessel can be used as the "effective" solubility of the formulated drug. If results from experiments in biorelevant *in vitro* setups mimicking drug transfer in the fasted state are available, the supersaturation/precipitation kinetic parameters can be derived from these setups (or used as initial estimates). If no data is available from experiments replicating the shift from the gastric to intestinal environment, or if estimations in fed state are to be made, the supersaturation ratio may be estimated from a 'simple' dissolution experiment by dividing the highest observed concentration by the concentration after 24 h, with both values derived from the same vessel and experiment. The rate of precipitation may be estimated from the duration in which the concentration in the dissolution vessel decreases from the maximum observed concentration to the concentration after 24 h.

Until now, PBPK models which aim to address questions regarding the absorption of a compound have relied on the equilibrium solubility of the crystalline drug as the input parameter to describe solubility in the GI tract. However, the equilibrium solubility of the crystalline drug substance may be far lower than the *in vivo* solubility of a drug substance if it is formulated as a bio-enabling formulation, and, depending on drug's precipitation propensity, it can result in a substantial underprediction of absorption, as demonstrated by the two case examples investigated in this work. Similar behaviour has also been reported by Emami-Riedmaier *et al*. for the ASD of venetoclax.[22]

While the use of this methodology enabled an estimation of the *in vivo* behaviour of the investigated nanosized and ASD bio-enabling formulations, there remains a significant gap in the knowledge relating to the *in vitro* characterisation and *in vivo* behaviour of these bioenabling formulations. For example, the *in vitro* characterisation of an ASD can be quite complex, due to the specific kinetics of supersaturation and precipitation behaviour. This behaviour may be reliant on interactions between the amorphous drug substance and the constituents of the biorelevant media, which is employed in the *in vitro* testing when characterising the ASD.[25–27] Therefore, in the cases where solubilisation by native surfactants rather than pH plays a key part in determining the *in vivo* behaviour of a formulated drug, the various kinds of surfactants used in the different varieties of biorelevant media may lead to different solubilisation behaviours. Further research is needed to improve our understanding in this area. Finally, validation of existing *in vitro* methods and *s*etups which simulate the transfer of drug from the stomach to the upper small intestine, is of great importance for the resulting *in silico* models.

3. Novel *in vitro* biopharmaceutics tools

3.1 Biorelevant media simulating porcine GI luminal conditions and their usefulness

Human intestinal fluids have been well characterised[28] and, based on these physiological characteristics, media to simulate human gastrointestinal contents have been developed. These so-called biorelevant media reflect either gastric (fasted state simulated gastric fluid, FaSSGF) or intestinal (fasted state simulated intestinal fluid, FaSSIF) conditions.[29,30] Biorelevant media simulating the environment in the intestine have proved to be a useful tool for humans as a reliable *in vitro* test and are commonly used for *in vitro* solubility screening. Furthermore, these established biorelevant media are routinely used for dissolution studies during drug development.

While these media have proved suitable for the evaluation of drug (intraluminal) performance in humans, FaSSIF does not reflect the composition of intestinal contents in conditions in preclinical models, due to critical differences between animal and human intestinal fluids. A porcine biorelevant medium was established based on characterisation of fasted intestinal porcine fluids (FaSSIFp). The medium is specially adapted to species specific characteristics in the intestine, such as pH, buffer capacity, osmolality, surface tension, bile and phospholipid concentration, with a summary of the composition of porcine biorelevant medium shown in Table 1.[31] FaSSIFp consists of phospholipids (PL) and bile salts (BS) at the physiological ratio of 0.013 (mM/mM) with an effective

bile acid content of 14.97 mM (5.25 mM sodium taurocholate and 9.72 mM sodium taurodeoxycholate) and a PL concentration of 0.20 mM (lecithin). Compared to FaSSIF, which contains taurocholates and PLs at concentrations of 3 mM and 0.75 mM, respectively, FaSSIFp has a 5.0-fold greater BS and a 3.8-fold smaller PL concentration. Relative to FaSSIF V2, where the PL concentration had been decreased to 0.2 mM, a 5.0-fold greater BS concentration still remains for FaSSIFp, but with the same PL content as FaSSIF V2. In comparison to FaSSIF V3, which contains a BS (taurocholate and glycocholate) and a PL concentration of 2.8 mM and 0.07 mM, respectively, FaSSIFp has a 5.3-fold greater BS and a 2.9-fold greater PL concentration. These key differences between human and porcine biorelevant media can influence the solubility behaviour of poorly soluble drug substances.

To assess suitability of FaSSIFp, Henze and co-workers compared the solubility of six model compounds in FaSSIFp to those observed using porcine intestinal fluids (PIF). The study demonstrated that the novel porcine biorelevant medium could reliably predict the solubility in PIF for the selected model compounds. The relationship between the solubility in PIF and FaSSIFp, was compared against the usage of human biorelevant media (FaSSIF) to forecast the *in vivo* solubility of the model drugs (Figure 1). Overall, FaSSIFp provided an greatly improved prediction of the solubility of the model drugs in PIF relative to human biorelevant media.

The key advantages of a species-specific simulated intestinal fluid are to enhance the evaluation of preclinical results using *in vitro* dissolution studies, and to allow for more reliable linkage between preclinical and clinical scenarios. Given the increasing prevalence of the pig model in preclinical drug development evaluation,[32] this study is therefore timely. In addition, the study has further advanced previous reports[33] that combine biorelevant species specific *in vitro* testing with *in silico* modelling. The merits of this combined *in vitro-in-silico* methodology in a preclinical setting are that it facilitates earlier predictions on the drug product performance after oral administration and subsequent studies in animal models become less exploratory and more confirmatory of the likely impact *in vivo*.[33] For example, by combining solubility and dissolution data in PIF and FaSSIFp respectively, with a porcine *in silico* PBPK model, reliable prospective simulations were obtained for the food effect observed in preclinical studies with venetoclax. A key impact of these advances is that while preclinical studies remain essential tools to explore *in vivo* pharmacokinetics (PK) of new drug substances, using a species-specific *in vitro-in silico* approach allows strategies to address/overcome low/variable bioavailability via formulation approaches to be explored earlier in the drug development process.[34]

3.2 Small – scale methodologies based on a two – stage biphasic system and a dissolution – permeation system for evaluating supersaturation and precipitation in upper small intestine in early pharmaceutical development

Supersaturation and precipitation in the intestinal lumen can greatly affect the oral bioavailability of a drug.[35] Weakly basic drugs and bio-enabling formulations have a propensity to be supersaturated in the small intestine. However, this state is not thermodynamically stable, which can lead to precipitation of the drug substance.[36] Due to the cost, time and ethical issues surrounding the use of both human and animal *in vivo* studies, it is important to develop reliable *in vitro* methods to evaluate supersaturation and precipitation in the upper intestinal lumen. In the early stages of development, when the availability of the drug substance and/or dose units are limited, full scale methods may not be applicable. Therefore, small-scale methods to evaluate the dynamic drug dissolution process in the upper GI tract are also needed.

Many small-scale methods have been proposed to evaluate drug precipitation in the upper intestinal lumen.[37] However, a lot of these approaches have significant disadvantages which limit their biorelevance i.e., failing to incorporate the intestinal drug absorption and / or not using Level II biorelevant dissolution media. In addition, many of the developed small-scale methods have not been tested using bio-enabling formulations, which are frequently employed in modern drug development as a result of the low aqueous solubility of numerous new drug candidates.[38] Finally, incorporation of supersaturation and precipitation results from small-scale experiments into PBPK modelling software has not been completed for many of the developed methods, which hampers the application of model-informed drug development during the early stages of development.

A new two-stage small-scale biphasic dissolution method was proposed (Figure 2).[39,40] Intestinal drug absorption was simulated by partitioning of drug from the aqueous phase into the decanol layer. Precipitation information taken from this setup was incorporated into PBPK modelling software and modelled average plasma profiles were evaluated relative to results reported from clinical studies. This biphasic methodology was also adapted to test the behaviour of lipid-based formulations (LBFs).[41] To achieve suitable dispersion of the LBF, a higher stirring speed was implemented during the gastric sector (300 vs. 100 rpm). In addition, pancreatic extract was added during the switch to intestinal conditions to replicate the digestion of the LBFs in the GI tract. A two-stage small-scale dissolution-permeation (D-P) method was also developed (Figure 3).[39] This method used transfer across a biomimetic membrane from the donor to acceptor chamber to simulate the intestinal absorption of drug.

Combining precipitation values calculated from the biphasic dissolution testing with PBPK modelling allowed for an improved representation of the average plasma profile for weakly basic drugs, relative to modelling using the default precipitation values.[39] In particular, this combination helped to understand the performance of two bio-enabling itraconazole products, an ASD and a cyclodextrin solubilised oral solution. The average modelled plasma profile for the ASD was not sensitive to intestinal precipitation, whereas the oral solution was sensitive to luminal precipitation. Oral absorption of the ASD in the model was instead limited by the dissolution and solubility of the compound in the GI tract. However, despite the precipitation observed for the oral solution, it had a greater exposure in the model compared to the ASD. These findings were in-line with the duodenal aspirates results from the clinical study,[42] highlighting the potential of model-informed drug development during early drug development. Currently, supersaturation and precipitation data are predominantly incorporated into the model after full-scale testing is employed.[43,44] Some studies employ default simulator values for precipitation and supersaturation parameters, [45] overlooking the individual drug substance/ formulation properties.

The biphasic lipolysis model was found to produce an improved simulation of the *in vivo* behaviour of the LBFs relative to the pH stat experiments.[41] In addition, using *in situ* UV fibre optic dip probes to quantify drug concentration in the biphasic lipolysis setup, allowed for a superior throughput capacity relative to the pH stat setup. This higher throughput is particularly beneficial in early stage development when many prospective formulations are being tested. The pH-stat lipolysis method is the most widespread standard method for *in vitro* assessment of LBFs.[46] However, in contrast to the proposed biphasic lipolysis method, the pH stat lipolysis method lacks an absorptive sink and typically only simulates conditions mimicking the intestine, which hinders its prediction of the *in vivo* performance of LBFs.[47]

The D-P method was useful to forecast the rank-order of the tested bio-enabling formulations. Greater concentrations of itraconazole were found in the acceptor chamber for the oral solution compared to the ASD, which matched the rank-order from the clinical results.[42]

The rapid shift from the gastric to the intestinal environment was a limitation of both small-scale setups. This sudden shift was different to the more gradual gastric emptying rate observed *in vivo* or the first order gastric emptying rate used in PBPK models. [48,49]Accordingly, faster rates of precipitation are expected to occur in these *in vitro* setups. Further work is required to investigate

certain excipient-specific effects in the biphasic lipolysis system.[41] In particular, a high ethanol content in the formulations as a co-solvent was thought to have altered drug flux into the decanol layer, leading to discrepancies compared to *in vivo* results. The slow partition of drug across the biomimetic membrane in the D-P system limits its application to calculate precipitation rates for input into PBPK modelling software.[39] This slow flux results in drug remaining in a highly unstable supersaturated state in the donor chamber, leading to a faster precipitation rate. Nevertheless, rank ordering of formulation performance was successful. A further consideration is that as the membrane is *in situ* for the entire experimental duration, partition across the membrane can occur during the gastric sector for compounds which are unionised in the *in vitro* gastric conditions e.g., some weakly acid drugs. This could be circumvented by appropriate experimental design.

3.3 Designing *in vitro* methodologies for the evaluation of GI drug transfer process, after a standard high-calorie, high-fat meal

Most of the published data on drug disposition in the upper GI lumen in the fed state have been collected by using liquid meals for inducing fed state conditions.[12] Relevant protocols, including the time at which the drug product is dosed in relation to meal administration, deviate from the protocol suggested by regulatory authorities for the evaluation of drug product performance in the fed state.[13,14,50–52]

Unlike with gastric emptying of contents which initiates upon meal consumption,[53,54] conventional tablets and capsules must disintegrate for emptying to initiate. Aspiration studies, after the standard meal,[55] have confirmed earlier imaging data in humans[56,57] according to which disintegration times of conventional tablets can be significantly extended when administered after the standard meal, up to about one hour. For hard gelatine capsules, shell rupture times are shorter, as times slightly longer than 10 min have been reported.[58] Published data on the drug transfer process from the stomach into the duodenum when immediate release tablets or capsules are administered after a high-calorie, high-fat meal have been very limited.[56]

Based on data collected within the PEARRL project,[59] the GI drug transfer process after disintegration of immediate release products of paracetamol (BCS Class I drug) and danazol (BCS Class II drug), administered as suggested by the regulatory authorities for bioavailability/bioequivalence studies, after administration of the high-calorie, high-fat standard meal,[13,14] is an apparent first-order kinetic process, with slower emptying rates than in the fasted state. Estimated gastric emptying half-lives were about 40 min i.e., about three times higher than in the fasted state, after drug administration with a glass of water.[60]

4 Optimised design of *in vivo* studies for investigating food effects

4.1 Standardised porcine model to predict food effects

Preclinical bioavailability predictions of new drug candidates require a thorough understanding of rate limiting steps in drug absorption. A key consideration in formulation development, particularly for BCS Class II and IV drugs, is the biopharmaceutical understanding of potential food effects of a selected bio-enabling formulation approach.[61] It is essential to assess the potential influence of food on oral bioavailability as soon as possible in the development process to facilitate optimal formulation design and avoid costly reformulation at a later stage.[62] While dogs have been traditionally used to investigate food effects in a preclinical stage,[63] recent studies have demonstrated the suitability of the pig model to forecast food-dependent bioavailability.[34,64] Henze and co-workers developed a standardised food effect protocol in the landrace pig which successfully predicted the food effect of fenofibrate, a BCS class II compound,[64] as well as for Venetoclax, a BCS class IV compound. In clinical food effect studies, a high-caloric, high-fat standard meal (FDA breakfast containing ~14kcal/kg body weight) is widely recommended by regulatory agencies such as the FDA and EMA, to robustly assess the impact of food on oral drug product performance.[50,65] In common with a previously reported canine food effect protocol, a high fat FDA style breakfast, containing eggs, toast, milk etc., was employed.[63] However in contrast to studies in dogs, the meal was not homogenised prior to the administration, and therefore the meal consistency /viscosity characteristics more closely matched the meal that is used in human studies. While previous attempts to establish a food effect protocol in minipigs failed to demonstrate a significant food effect for atazanavir (positive food effect) and pravastatin (negative food effect),[66] Henze *et al.* employed a higher caloric meal (26kcal/kg versus ~ 16 kcal/kg bodyweight). This underlines the relevance of a standardised protocol which takes into consideration that the caloric content in the fed state must be greater in pigs in order to simulate the effects of food in humans. While the reported studies by Henze *et al.* are promising in establishing conditions, including fasting regime, amount and type of food etc. for landrace pigs, further studies are required to improve the knowledge on the pig model to predict food dependent bioavailability of drugs, using a wider range of drugs with both positive, as well as negative reported food effects.

4.2 Designing the most appropriate drug dosing conditions to adults for a reliable extrapolation of drug exposure data to infants

According to current development timelines, paediatric drug product development and formulation evaluation usually commence after the early clinical phases in adults.[67,68] As in adults, across all paediatric age ranges the oral route of administration is favoured. As a result, understanding of the absorption process and effect of food on drug PK as function of age is essential for paediatric drug (formulation) development.[67,68] The investigation of drug performance under fed conditions might be even more important in the youngest paediatric subpopulations (neonates birth – 28 days and infants 1 month – 2 years of age), who receive frequent feedings and are thus rarely fasted. [68] Due to ethical issues, the number of PK studies, especially food effect studies, in infants are limited and food effect investigations for the paediatric formulations are commonly completed in adults.[68] A comparison of studies found in the literature revealed disparity in outcomes between food effect investigations in infants/young children (2 - 42 months) in which fed conditions were induced using infant formula[69,70] versus food effect investigations performed in adults using a standard meal to investigate the same drug.[71] According to regulatory guidelines, food effect studies for adults are designed to induce maximum effect of the GI physiology by employing a high-fat, high calorie meal.[13,72] For paediatric formulations, new draft regulatory guidelines propose the "use of foods and quantities of food that are commonly consumed with drugs in a particular paediatric population" for evaluation in adults i.e., infant formula for drug formulations to be administered to infants.[13] Additionally, if "the same to-be-marketed formulation that is approved for use in adults is approved for use in a paediatric population, a separate food effect study is not necessary."[13] Ultimately, the results from the food effect study are to be extrapolated to the paediatric population of interest i.e., via PBPK modelling. Based on the ambiguity of the food effect result comparison between studies in infants and adults employing different meals and the lack of food effect studies for infant formulations in adults employing age-relevant meals, a food effect study designed to evaluate paediatric drug formulations under age-appropriate conditions is required to understand food influence on drug performance.[71]

An innovative design for investigations of relative bioavailability using healthy adult volunteers was developed and applied for the evaluation of formulations that are to be administered to the infant paediatric (sub)population i.e., 1 month – 2 years.[71] Within the proposed design, the infant formulations were tested on a crossover basis under different prandial and dosing conditions in healthy adult subjects (Figure 4): under fasted state conditions as defined by regulatory agencies (fasted conditions), under fed state conditions as defined by regulatory agencies (fed conditions), and

under conditions mimicking dosing in infants (infant fed conditions). Panadol® suspension containing paracetamol (BCS class I) and Nurofen® suspension containing ibuprofen (BCS class II) were selected as model drug formulations due to their unproblematic absorption and lack of luminal degradation.[68] With the proposed study design, the infant meal used for simulation of infant fed conditions exhibits a different caloric breakdown, lower calorific density, and a homogeneous liquid texture compared to the recommended solid-liquid meals recommended for food effect studies in the development of adult medicines.

For both drugs, their absorption was delayed when administered during infant formula consumption compared to the fasted state, while fed conditions established with the standard adult meal did not significantly delay absorption compared to the fasted state.[71] The different types of meal textures employed in the study might result in different mixing of the meal types with the liquid paediatric formulations, leading to differences in drug stomach emptying and appearance in the small intestine and subsequent drug absorption. Under fed conditions drug (formulation) emptying from the stomach occurred independently from the standard adult meal, whereas under infant fed conditions drug (formulation) emptying occurred predominantly with the gastric contents. For drugs without limitations relating to their permeability, which have a high solubility in the environment of the intestinal lumen, have no noted intraluminal interactions with food and were administered as conventional dosage forms (aqueous suspensions), it appears that the effects of food on drug absorption in infants cannot be suitably assessed using the methods proposed by regulatory agencies for testing food effects of adult drug products.

Data from the three different dosing conditions were used to extrapolate the exposure from adults to infants after oral administration of the paediatric aqueous suspensions using observed data in the target population. [73–75] For this reason, a PBPK model for adults was created and evaluated, followed by model scaling to infants using the PBPK modelling platform GastroPlus™ 9.7 (Simulations Plus, Lancaster, CA). The extrapolated fasted and infant fed conditions appeared to be more useful to describe early drug exposure in infants, while extrapolation of the fed conditions (induced by the standard high-calorie, high-fat meal) failed to capture drug absorption in infants.[73,74] The *in silico* results confirm the usefulness of the proposed methodology, while suggesting that caution is needed when using data following the standard meal to evaluate paediatric drug formulations for infants.

The usefulness of the proposed methodology is currently limited to highly absorbable drugs, while the implications for drugs with lower permeability and/or active uptake have not yet been studied.

Additionally, a larger range of drugs with different physicochemical characteristics and various formulation types with different excipients need to be investigated to widen the scope of the current findings. Vague definition and occurrence of a fasted state in infants, as well as differences in the physiologies between adults and infants might hamper comparison between these age groups. Coupling the *in vivo* data in adults with *in silico* tools, such as PBPK modelling, might be a useful way forward to enable further mechanistic understanding and extrapolation of the influence of different drug dosing conditions to infants, followed by evaluation using observed paediatric clinical data. Paucity of PK data in infants under fasted and/or fed conditions along with poor study reporting of drug formulations, dosing conditions, and age-group stratification pose a challenge to verify the usefulness of the data acquired in adults. To improve current tools and methodologies, more well-designed studies in the various paediatric age-groups addressing the current knowledge gaps are needed.[68]

5 Conclusion

Based on the PEARRL project results, optimisation of current *in vitro* biopharmaceutics tools is proposed. In addition, new *in vitro* tools have been developed to meet current gaps in the biopharmaceutics 'toolkit'. Finally, advice on the appropriate design of preclinical and clinical studies has been provided. It is expected that these developments will facilitate the oral drug product development process and the regulation of new oral drug products.

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Tables:

Table 1: Composition and physicochemical properties of porcine (FaSSIFp) biorelevant media.[31]

FaSSIFp		
Composition		[mM]
Buffer	Sodium dihydrogen phosphate	35.71
	Sodium hydroxide	13.62
	Sodium chloride	135.32
BS	Sodium taurocholate	5.25
	Sodium taurodeoxycholate	9.72
PL	Lecithin	0.20
FA	Sodium oleate	2.82
Characteristics		
pH	7.0	
Buffer capacity	19.4 [mmol $l^{-1}\Delta pH^{-1}$]	
Osmolality	387 [mOsm kg^{-1}]	

BS: Bile Salts; PL: Phospholipids; FA: Fatty Acids;

Figures:

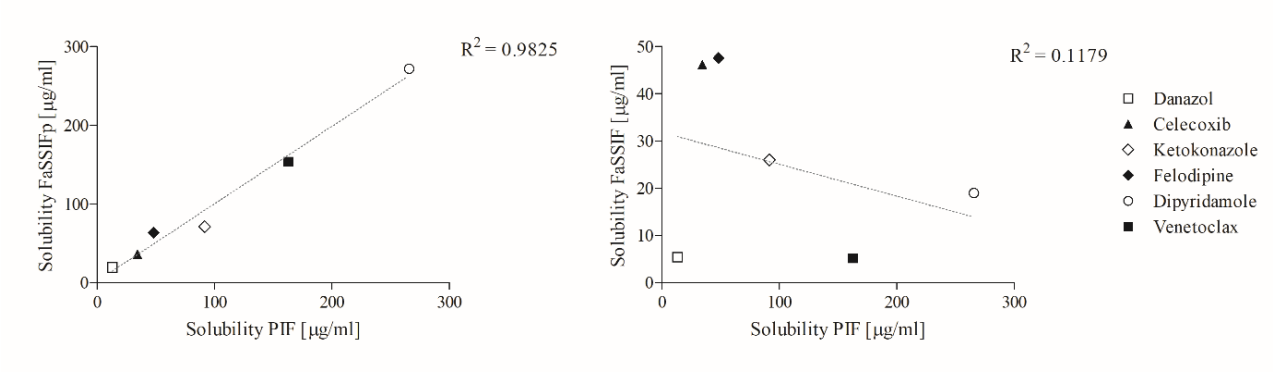

Figure 1. A: Correlation of the solubility of six model compounds in pig intestinal fluids (PIF) and in porcine Fasted State Simulated Intestinal Fluid (FaSSIFp); B: Correlation of solubility of six model compounds in pig intestinal fluids (PIF) and human Fasted State Simulated Intestinal Fluid (FaSSIF). Adapted from Henze at al.[31]

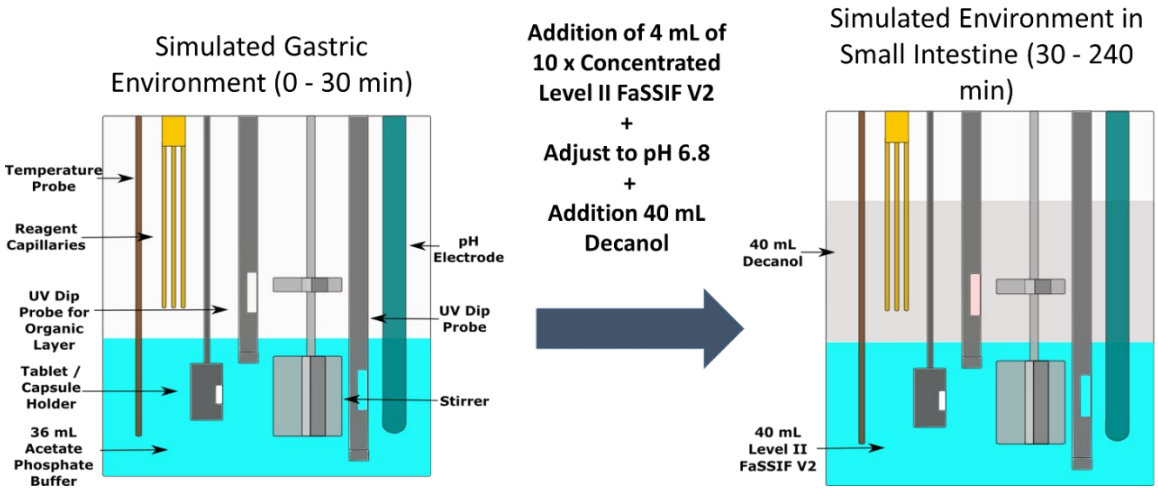

Figure 2: Schematic of the biphasic dissolution test using the inForm platform. Adapted from O'Dwyer et al. [39]

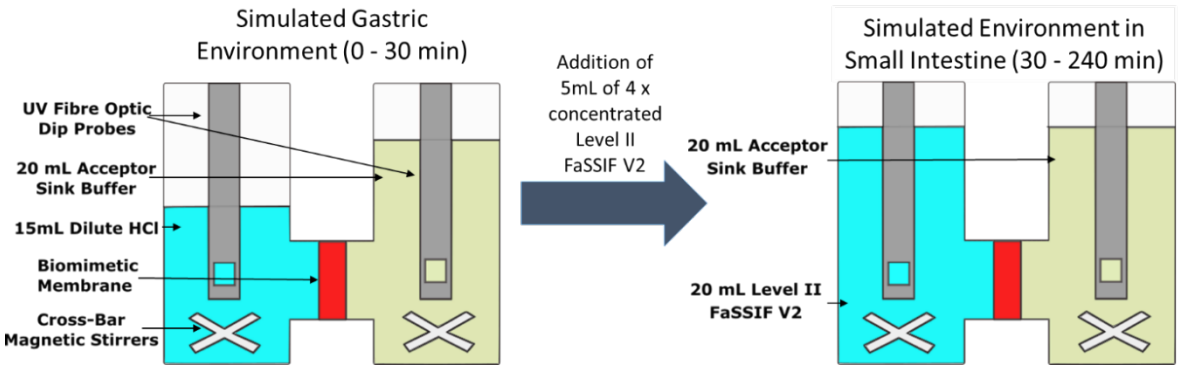

Figure 3: Schematic of the dissolution-permeation (D-P) test using the µFLUX apparatus. Adapted from O'Dwyer et al.[39]

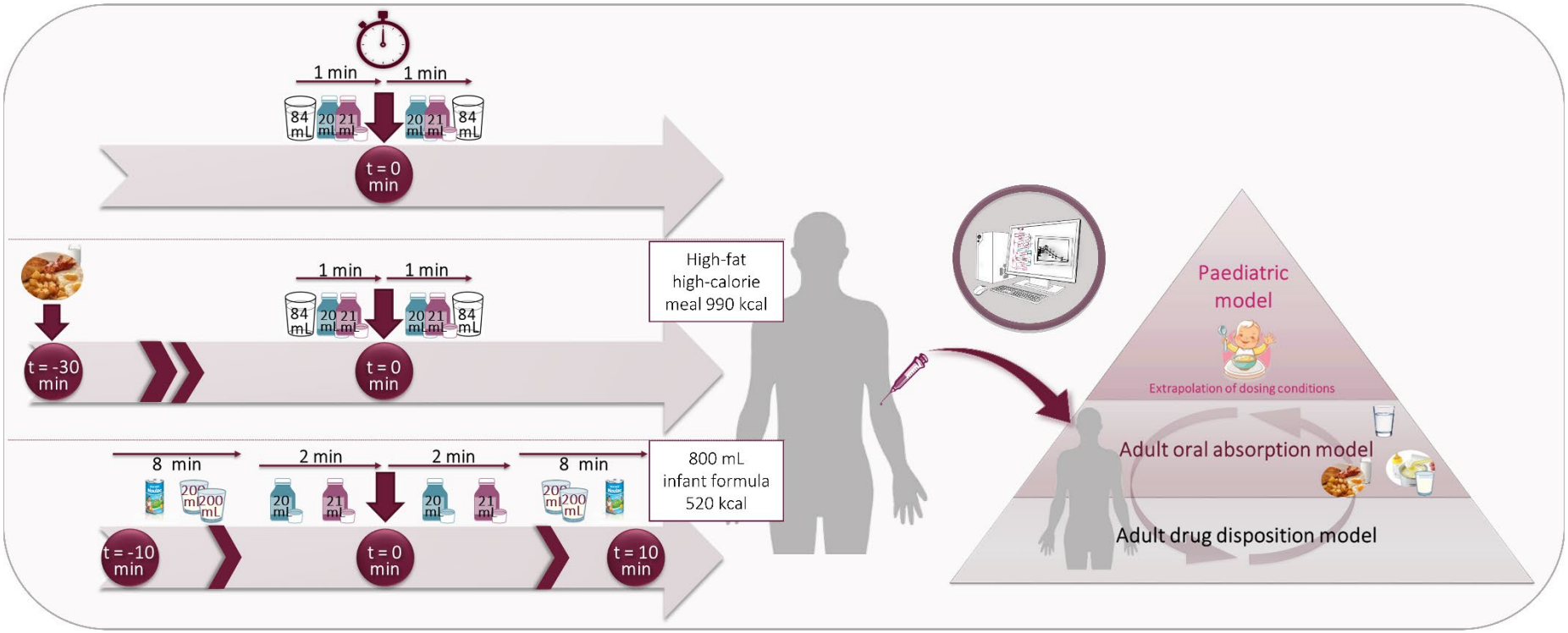

Figure 4: Graphical depiction of the applied methodology for infant drug formulation evaluation involving an in vivo-in silico investigation, i.e. relative bioavailability study design for food effect evaluation of infant drug formulations employing an age-appropriate meal and the standard high-calorie, high-fat meal in healthy adult volunteers with subsequent PBPK modelling and evaluation with in vivo data in infants. Adapted from Statelova et al.[71,75]