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Review

PBPK models for the prediction of *in vivo* performance of oral dosage forms

Edmund S. Kostewicz [a,*], Leon Aarons [b], Martin Bergstrand [c], Michael B. Bolger [d], Aleksandra Galetin [b], Oliver Hatley [b], Masoud Jamei [e], Richard Lloyd [f], Xavier Pepin [g], Amin Rostami-Hodjegan [b,e], Erik Sjögren [h], Christer Tannergren [i], David B. Turner [e], Christian Wagner [a], Werner Weitschies [j], Jennifer Dressman [a]

[a] *Institute of Pharmaceutical Technology, Goethe University, Frankfurt/Main, Germany*
[b] *Centre for Applied Pharmacokinetic Research, Manchester Pharmacy School, The University of Manchester, United Kingdom*
[c] *Pharmacometrics Research Group, Department of Pharmaceutical Biosciences, Uppsala University, Sweden*
[d] *Simulations Plus, Inc., Lancaster, CA, United States*
[e] *Simcyp Limited (a Certara Company), Blades Enterprise Centre, Sheffield, United Kingdom*
[f] *Department of Drug Metabolism and Pharmacokinetics, GlaxoSmithKline, Ware, Hertfordshire, United Kingdom*
[g] *Department of Biopharmaceutics, Pharmaceutical Sciences R&D, Sanofi, Vitry sur Seine Cedex, France*
[h] *Department of Pharmacy, Uppsala University, Uppsala, Sweden*
[i] *Medicines Evaluation CVGI, Pharmaceutical Development, AstraZeneca R&D Mölndal, Sweden*
[j] *Department of Biopharmaceutics, University of Greifswald, Greifswald, Germany*

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ABSTRACT

Drug absorption from the gastrointestinal (GI) tract is a highly complex process dependent upon numerous factors including the physicochemical properties of the drug, characteristics of the formulation and interplay with the underlying physiological properties of the GI tract. The ability to accurately predict oral drug absorption during drug product development is becoming more relevant given the current challenges facing the pharmaceutical industry.

Physiologically-based pharmacokinetic (PBPK) modeling provides an approach that enables the plasma concentration–time profiles to be predicted from preclinical *in vitro* and *in vivo* data and can thus provide a valuable resource to support decisions at various stages of the drug development process. Whilst there have been quite a few successes with PBPK models identifying key issues in the development of new drugs *in vivo*, there are still many aspects that need to be addressed in order to maximize the utility of the PBPK models to predict drug absorption, including improving our understanding of conditions in the lower small intestine and colon, taking the influence of disease on GI physiology into account and further exploring the reasons behind population variability. Importantly, there is also a need to create more appropriate *in vitro* models for testing dosage form performance and to streamline data input from these into the PBPK models.

As part of the Oral Biopharmaceutical Tools (OrBiTo) project, this review provides a summary of the current status of PBPK models available. The current challenges in PBPK set-ups for oral drug absorption including the composition of GI luminal contents, transit and hydrodynamics, permeability and intestinal wall metabolism are discussed in detail. Further, the challenges regarding the appropriate integration of results from *in vitro* models, such as consideration of appropriate integration/estimation of solubility and the complexity of the *in vitro* release and precipitation data, are also highlighted as important steps to advancing the application of PBPK models in drug development.

Abbreviations: ABL, Aqueous Boundary Layer; BCS, Biopharmaceutics Classification System; BDDCS, Biopharmaceutical Drug Disposition Classification System; BE, Bioequivalence; CYP, Cytochrome P450; P_{eff}, effective permeability; EP, European Pharmacopoeia; ER, Extended Release; FE, Fold Error; GI, Gastrointestinal; IR, Immediate Release; IVIVC, *in vitro–in vivo* Correlation; MMC, Myoelectric Motor Complex; MR, Modified Release; OrBiTo, Oral Biopharmaceutical Tools; BA, Oral Bioavailability; PBPK, Physiologically Based Pharmacokinetic Modeling; QbD, Quality by Design; QC, Quality Control; UGT, UDP Glucuronosyltransferase; FDA, US Food and Drug Administration; USP, United States Pharmacopeia.

* Corresponding author. Address: Institute of Pharmaceutical Technology, Goethe University, Max-von-Laue Str. 9, 60438 Frankfurt/Main, Germany. Tel.: +49 69 798 296 92; fax: +49 69 798 296 94.
E-mail address: kostewicz@em.uni-frankfurt.de (E.S. Kostewicz).

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It is expected that the "innovative" integration of *in vitro* data from more appropriate *in vitro* models and the enhancement of the GI physiology component of PBPK models, arising from the OrBiTo project, will lead to a significant enhancement in the ability of PBPK models to successfully predict oral drug absorption and advance their role in preclinical and clinical development, as well as for regulatory applications.

Contents

1. Introduction

Physiologically-based pharmacokinetic (PBPK) models traditionally employ what is commonly known as a "bottom-up" approach. The concept is to describe the concentration profile of a drug in various tissues as well as in the blood over time, based on the drug characteristics, site and means of administration and the physiological processes to which the drug is subjected. Thereby, PBPK modeling takes into account the factors influencing the absorption, distribution and elimination processes (Rowland et al., 2011). In PBPK modeling, parameters are determined *a priori* from *in vitro* experiments and the physiology, utilizing *in silico* predictions to predict *in vivo* data. In one of the earliest invocations of the PBPK approach, a Swedish physiologist and biophysicist, Teorell, developed a five compartment scheme to reflect the circulatory system, a drug depot, fluid volume, kidney elimination and tissue inactivation (Teorell, 1937a,b). The next advances came over 20 years later, when Edelman and Liebmann recognized that the total body water was not equally accessible, but rather should be divided into plasma, interstitial-lymph, dense connective tissue and cartilage, inaccessible bone water, transcellular and intracellular components (Edelmann and Liebmann, 1959). A few years later, physiological models were introduced to describe the handling of drugs by the artificial kidney as well as to describe the pharmacokinetics of thiopental and methotrexate (Bischoff and Dedrick, 1968; Bischoff et al., 1971; Dedrick and Bischoff, 1968). In the early years however, *in silico* predictions were hampered by lack of capacity in computing as well as large gaps in physiological knowledge. Further, the design of *in vitro* experiments, particularly in the area of predicting drug release, transport and metabolism, was still at a rather rudimentary stage. As knowledge in these areas grew, and more powerful computers became commonplace, it was possible to create better PBPK models and they became more widely used, such that for example, in 1979, a review of PBPK models for anticancer drugs was published (Chen and Gross, 1979). As early as 1981, the brilliant pharmacokineticist, John Wagner, foresaw applications of pharmacokinetics to patient care such as individualization of patient dose and dosage regimen, determination of the mechanism of drug–drug interactions, prediction of pharmacokinetics of drugs in man from results obtained in animals using physiologically based models, development of sophisticated computer programs to obtain population estimates of pharmacokinetic parameters and their variabilities, therapeutic monitoring and prediction of the time course of the intensity of pharmacological effects. In the meantime, many of these ideas have been turned into reality, or are being turned into reality, through the application of PBPK models (Wagner, 1981).

The first commercial software to attempt a comprehensive description of the gastrointestinal (GI) tract in the context of a PBPK model was GastroPlus™. The first version, introduced in 1998, used a mixing-tanks-in-series approach to describe the movement of drug from one region in the GI tract to the next, with simple estimations of dissolution based on aqueous solubility and absorption rate constants based on existing pharmacokinetic data. Even at this stage, it was possible to obtain a reading on whether absorption (uptake across the GI mucosa) or solubility/dissolution

would be limiting to the drug's bioavailability. This was already a very significant advance for scientists working in formulation development, as it enabled goals for the formulation to be set on a more realistic basis, recognizing that solubility and dissolution problems are much more amenable to formulation solutions than permeability limitations.

In addition to GastroPlus™, several other commercial PBPK models such as Simcyp and PK-Sim® now have evolved descriptions of the GI tract. Additionally, there are some software programs available which can be tailored to predict *in vivo* performance of oral formulations, including MATLAB® and STELLA® – these tend to be favored by academic groups. In the industry, as well as utilizing the commercially available PBPK models, some companies have "home-grown" models tailored to the specific needs of their development programmes. All of these programmes now strive to account for all relevant processes to the GI absorption of drugs, including release from the dosage form, decomposition/complexation in the GI tract, the various mechanisms of drug uptake and efflux and first pass metabolism, whether this be in the gut wall or liver, and to describe the interplay of these factors in determining the rate and extent of drug absorption from the GI tract.

As part of the **Or**al **Bi**opharmaceutical **To**ols (OrBiTo) project within the **I**nnovative **M**edicines **I**nitiative (IMI) framework, this review provides a summary of the current status of PBPK models available for predicting the *in vitro* performance of orally administered drugs and their formulations. The current challenges in PBPK set-ups for oral drug absorption including the composition of GI luminal contents, transit and hydrodynamics, permeability and intestinal wall metabolism are then discussed in detail. Further, the challenges regarding the appropriate integration of results from *in vitro* models, such as consideration of appropriate integration/estimation of solubility and the complexity of the *in vitro* release and precipitation data, are also highlighted as important steps to advancing the application of PBPK models in drug development.

2. Current PBPK models and how they handle GI absorption

Many drug companies are now building PBPK models for all new candidate drugs early in the discovery and development cycles. These models can be parameterized using *in silico* (distribution) and *in vitro* (intrinsic clearance) methods and can provide a "ballpark" estimate of the human and/or animal plasma concentration vs. time profile prior to *in vivo* testing in animals. Furthermore, they provide one of the most successful methods for scale-up from animals to human. PBPK models also allow for an early estimate of local organ tissue concentrations which can be tied to pharmacodynamic models to get an estimate of the response in any particular tissue.

Whilst the use of PBPK models by the pharmaceutical industry as both a prediction and mechanistic analysis tool to gain a holistic analysis of drug absorption is fairly widespread, as described in the following section the structure of different commercially available PBPK models can vary considerably.

2.1. The Simcyp Simulator

The Advanced Dissolution Absorption and Metabolism (ADAM) model (Jamei et al., 2009b) is a multi-compartmental GI transit model fully integrated into the Simcyp human population-based Simulator (Jamei et al., 2009a,b) as well as the rat, mouse and dog simulators. The Simulator provides both pharmacokinetic and pharmacodynamics models and a separate pediatric module.

The ADAM model treats the GI tract as one stomach, seven small intestine and one colon compartment(s) (Fig. 1), within each of which, drug can exist in several states simultaneously viz. unreleased, undissolved (solid particles), dissolved or degraded. Dissolved drug is permitted to attain supersaturated levels controlled by the maximal allowable extent of supersaturation, its duration and first order precipitation rate constants. Drug can be dosed in a supersaturated state or supersaturation may be attained as a consequence of solubility change when moving from one region of the GI tract to another. The most well-known example of this phenomenon is when the pH-dependent solubility of a weak base with $pK_a < 7$ is reduced significantly upon transit from the fasted stomach to the duodenum. ADAM includes the population mean and inter-individual variability of regional luminal pH and bile salt concentrations in the fasted and fed states. It models the interplay of these factors upon solubility and dissolution rate (Persson et al., 2005a) via a bile micelle solubilization (Glomme et al., 2007) and a diffusion layer (Jamei et al., 2009b; Patel et al., 2014) model.

Separate age-dependent functions define the (a) return of elevated fed stomach pH to basal (fasted) levels, and (b) population-specific fractions of achlorhydric individuals. A general North European Caucasian population has a fixed proportion (8%) of achlorhydric subjects while the Simcyp Japanese population has proportions that increase with age (for more detail see Jamei et al., 2009b). Differences such as these are captured in the various ethnic and disease populations available within the Simulator. The liver cirrhosis libraries, for example, define three levels of severity of disease and capture extended gastric emptying times, decreases in gut Cytochrome P450 (CYP) 3A4, decreases in portal blood flow, increases in villous blood flow and lower levels of plasma binding proteins (Johnson et al., 2010). Also available are obesity and renal impairment disease libraries, a pediatric library (with built-in parameter age-dependencies as far as they have been quantified) and Japanese and Chinese ethnic libraries. When running a simulation the appropriate population should be selected and then the trial size (numbers and groups) together with age-range, gender proportions, fasted/fed status, fluid taken with dose and dosing regimen including staggering for up to four co-dosed drugs.

A time-dependent fluid volume dynamics model handles basal luminal fluid, additional fluid taken with dose, biological fluid secretion rates, and fluid absorption rate in the fasted or fed state. Gastric emptying, intestinal transit times and their inter-individual variability are incorporated for the fasted and fed states. The

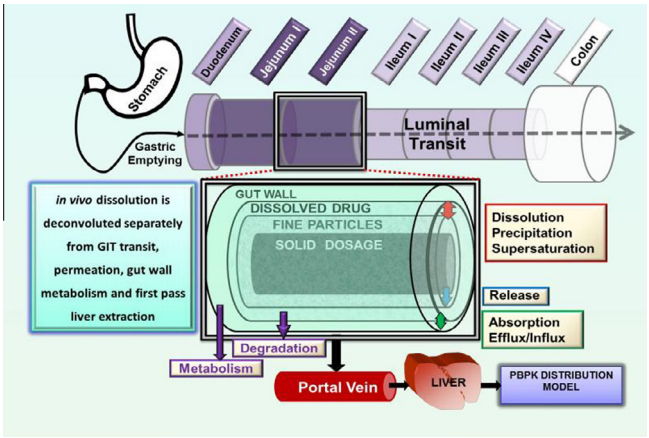

Fig. 1. Structure and features of the Simcyp ADAM Model: the relative lengths (diameters) of the cylinders reflect the relative lengths (or diameters) of the GI segments for a representative individual *in vivo* while the purple shading represents the CYP 3A enzyme abundance distribution in the intestinal tract.

lengths and radii of intestinal regions are individualized based upon covariation with body surface area and jejunal–ileal ratios, enabling individualized regional absorptive surface areas to be determined (Jamei et al., 2009b).

Gut wall passive and active permeability ($P_{eff,man}$) can be predicted from *in vitro* permeability measurements (Caco-2, etc.) or using QSAR-type models; regional P_{eff} differences can be specified. The dog, mouse and rat models also include a mechanistic model that accounts for transcellular, paracellular and mucus/unstirred layer permeability, calculates regional absorptive surface area (SA) from villous dimensions (and in humans the plicae circulares), and further scales to effective SA by the method of Oliver et al. (1998). Bile micelle partition of drug affects free fraction in luminal fluids (Buckley et al., 2013) and thus can provide an additional food effect where bile salt concentrations are elevated particularly in the fed state.

Drug entering the enterocytes may be metabolised by gut wall enzymes and/or subject to efflux by gut wall transporters in a complex interplay (Darwich et al., 2010). Regional abundances of gut wall enzymes (CYPS: 2C9, 2C19, 2D6, 2J2, 3A4, 3A5) and transporters (P-glycoprotein (P-gp), MPRP2, BCRP) are incorporated where information is available (Harwood et al., 2012) and a generic apical influx transporter functionality is provided. Segregated villous blood flows to each compartment of the intestinal tract are scaled up 1.3-fold in the fed state to account for increased postprandial blood perfusion. Enterohepatic recirculation of drug is handled with different patterns of gallbladder accumulation or by-pass according to fasted or fed status. In the fasted state gallbladder drug release is linked to late Phase II/early Phase III of individualized Interdigestive Migrating Motor Complex (IMMC) cycle times. Metabolic and transporter-mediated drug–drug interactions in the intestinal wall can be modeled separately in each compartment (Neuhoff et al., 2013).

ADAM has been adapted to model the impact of bariatric surgery in morbidly obese patients on drug BA including Roux-en-Y gastric bypass, biliopancreatic diversion with duodenal switch, sleeve gastrectomy and jejunoileal bypass (Darwich et al., 2012). In the absence of clinical studies in such population groups these models provide useful guidance for adjusting dose regimens. In another application, Patel et al. (2014) described the prediction of formulation-specific food effects for three formulations of the Biopharmaceutics Classification System (BCS)/Bipharmaceutical Drug Disposition Classification System (BDDCS) Class 2 drug nifedipine using the ADAM model (Fig. 2). BCS/BDDCS classification and QSAR-based approaches failed in this regard because they consider drug properties alone rather than those of the formulation. The nifedipine ADAM model was based solely upon *in vitro* data, aside from prior knowledge of negligible renal clearance (pre-clinical species) and that nifedipine is readily absorbed from the colon.

The ADAM model can also be used to establish physiologically-based (PB) *in vitro–in vivo* correlations (IVIVC) (PB-IVIVCs). Traditional deconvolution methods are generally empirical and do not deconvolute *in vivo* dissolution separately from GI transit, permeation or first-pass metabolism. Where there is significant gut wall or hepatic first-pass metabolism of drug establishment of robust relationships between *in vitro* and deconvoluted *in vivo* dissolution profiles can become difficult, perhaps requiring complex non-linear functions. PBPK models separately handle the different mechanisms influencing drug disposition. Thus PB-IVIVC models should permit the development of improved/simplified IVIVCs for BCS I or II drugs with significant first pass extraction and inter-individual variability. It also opens up the possibility to develop IVIVCs for: (i) CR formulations of BCS III drugs (e.g. gastro-retentive) where absorption is governed by the complex interplay of release, transit/gastro-retention and permeability rather than being solely release-limited, and (ii) BCS IV compounds whose absorption may be controlled by a similar interplay of multiple processes. This approach has thus far been successfully applied to the development and validation of IVIVC for CR formulations of the BCS Class I, high first pass extraction (CYP 2D6 substrate) drug metoprolol and also employed in the design of new formulations and in understanding population variability (Patel et al., 2012a,b) (Fig. 3).

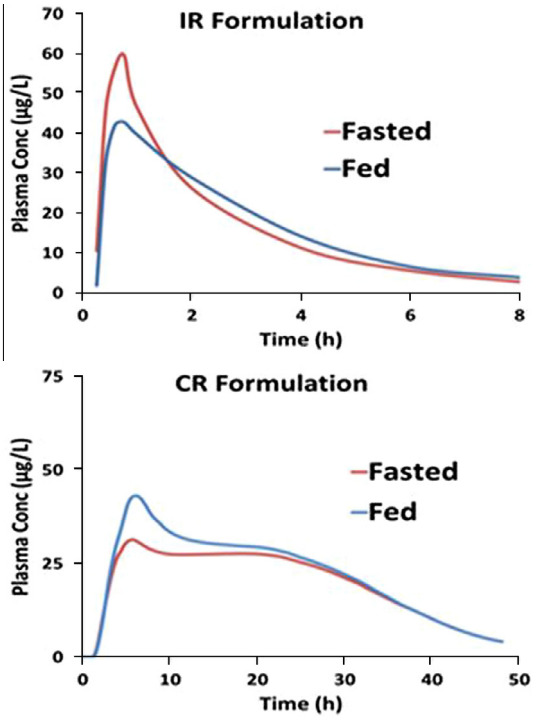

Fig. 2. Prediction of food effects on the PK profiles of nifedipine immediate and controlled release formulations (see Patel et al., 2014 in this issue of EJPS).

2.2. GastroPlus™

GastroPlus™ is a whole-body PBPK model distributed by Simulations Plus (Simulations Plus, Inc., Lancaster, CA, USA). Currently version 8 is available. GastroPlus™ uses the Advanced Compartmental Absorption Transit (ACAT™) mechanistic absorption model (Fig. 4) which has been described in the literature (Agoram et al., 2001) to model the absorption of oral formulations from the GI tract.

The GastroPlus™ implementation of PBPK provides an internal module called PEAR Physiology™ (Population Estimates for Age-Related Physiology) for the calculation of organ physiologies for American (Western) and Japanese (Asian) human models for any age from 0 to 85 years old and also will generate unique new populations using user-defined coefficients of variation for population simulations. The GastroPlus™ PBPKPlus™ module will automatically calculate tissue:plasma partition coefficients (K_p) or convenient import of user-specified K_p and f_{ut} values from tab-delimited ASCII text files. Beagle dog, rat, monkey, and mouse physiologies are also available for modeling preclinical *in vivo* data. Building models of the preclinical species can improve the confidence in estimation of input parameters for the human model. When *in vitro* or preclinical data are not available, model parameters can be fitted for individual tissues to best match observed data.

The ACAT model for oral absorption describes each of the following including: drug release from the formulation, solubility,

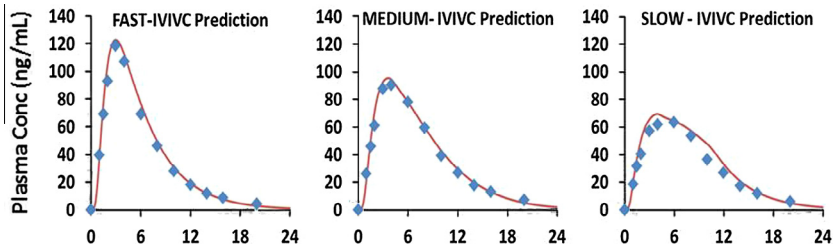

Fig. 3. Predicted profiles (lines) vs. observed (diamonds) for fast, medium and slow release formulations of metoprolol.

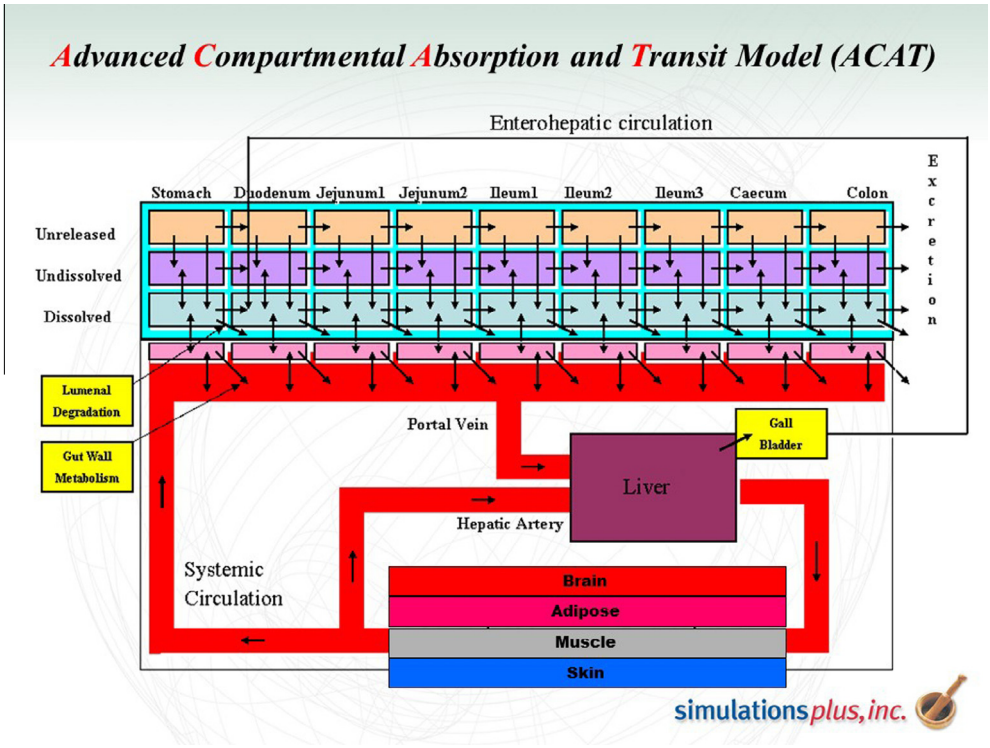

Fig. 4. Structure and features of the Advanced Compartmental Absorption Transit (ACAT™) Model.

dissolution/precipitation rate, chemical stability, permeability, carrier mediated influx and efflux and gut wall metabolism using differential equations, most of which are defined in the user manual. Most of the equations involve linear kinetics, whilst Michaelis–Menten nonlinear kinetics is used to describe saturable metabolism and carrier-mediated transport.

Input data are *in vitro, in vivo* or *in silico* estimates of the drug- or formulation-related parameters, e.g., drug aqueous solubility–pH relationship, permeability, particle size distribution and formulation type. Physiological parameters such as GI transit time, pH, absorptive surface area, bile salt concentrations in each compartment, pore size and density, compartment dimensions and fluid content, etc. are built into the model but can be modified by the user. Many of the input parameters required for the GastroPlus model can alternatively be predicted from structure using the optional ADMET Predictor™ module or the full ADMET Predictor program (Simulations Plus, Inc., Lancaster, CA, USA).

In GastroPlus™ (version 8) the formulation types that can be selected include both Immediate Release (IR) formulations (solution, suspension, tablet, and capsule) and Controlled Release (CR) formulations (enteric-coated or other form of Delayed Release (DR)). For CR, release of either dissolved material (drug in solution) or undissolved material (solid particles, which then dissolve according to the selected dissolution model) can be evoked. For CR formulations, percent released vs. time can be specified as tabular release-time entries or alternatively using a single or double Weibull function.

The drug must be in solution before it can be absorbed. The pK_a value(s) and the Solubility Factor (SF – the ratio of the solubility of the ionized to unionized forms) are used to predict a pH vs. solubility curve under aqueous conditions. This solubility is then corrected for the influence of bile salts based on a theoretical relationship or alternatively a directly measured *in vitro* solubility determined in biorelevant media can be used instead. The pK_a value(s) and SF can be fitted to measured solubility data in aqueous buffers. If the drug concentration in a compartment exceeds the solubility of drug in that compartment, the drug may precipitate. In this case, a drug may precipitate rapidly or may remain in a supersaturated state. However, the actual precipitation time is not easy to predict and may depend on the drug as well as the formulation used. GastroPlus™ uses single or multiple first order exponential precipitation or a complete mechanistic nucleation and growth model that can account for formulation effects due to nucleation inhibitors and solubilizers (Lindfors et al., 2008). When using the mechanistic nucleation and growth model, the simulation outputs include the size, time, and degree of

supersaturation for initial particle formation as well as the maximal degree of supersaturation achieved and the dynamics of particle size changes and absolute numbers in all of the GI compartments.

Since the influence of bile salts on solubility, diffusion coefficient, dissolution rate, and precipitation is well documented, GastroPlus™ automatically adjusts the concentration of bile salts in each compartment for fasted and fed states, with the bile being almost completely reabsorbed by the distal end of the small intestine. In the GastroPlus model, a solubilization ratio based on log*P* (Mithani et al., 1996) can be used for the drug if measured solubilities in biorelevant media (Fasted State Simulated Gastric Fluid (FaSSGF) and Fasted and Fed State Simulated Intestinal Fluid (FaSSIF and FeSSIF)) are not available. The solubilization ratio can also be predicted from measured solubility in FaSSGF, FaSSIF and FeSSIF. There are a number of choices for describing the dissolution kinetics, including the Nernst–Brunner modification of the Noyes–Whitney equation, Wang–Flanagang, Tekano's *Z*-factor and instantaneous dissolution model. The initial particle size can be described by a distribution (Normal and Log-Normal) or tabulated data to describe more complex particle size distributions. The solubility of very small particles (nanoparticles) may be increased via the Kelvin effect. The NanoFactor Effect is applied to the dissolution equation which effectively increases solubility (as well as concentration gradient to create faster absorption).

Loss of the drug via chemical/metabolic pH-dependent degradation in the lumen, e.g. valacyclovir hydrolyzed back to acyclovir at neutral pHs and above (Sinko and Balimane, 1998) is determined by interpolation from an input table of degradation rate (or half-life) vs. pH, and the pH in each compartment.

Absorption rate in the ACAT model depends on the effective permeability of the drug (transcellular and/or paracellular) and the physiological Absorption Scale Factor (ASF) for each compartment as well as the time-dependent concentration gradient between lumen and enterocyte (transcellular) or portal vein (paracellular). ASF is used to account for changes in surface-area-to-volume ratio along the GI tract, changes in distribution coefficient and regional permeability due to changes in pH, and changes in paracellular pore size and porosity. These factors also form the basis of a mechanistic model for scaling gut physiology between preclinical and human studies.

In addition, *in vitro* K_m values for influx transporters can be used to accurately simulate nonlinear dose dependence for substrates including valacyclovir, valganciclovir, gabapentin, and amoxicillin (Bolger, Lukacova et al., 2009).

Local variations in absorption/exsorption e.g. due to different expression of active influx or efflux transporter(s) are calculated using included distributions of various transporters from a number of publications. These are the relative expression levels of the transporter within the intestinal compartments and unlike enzymes, these values are not related to the liver.

Gut wall metabolism is based on local expression levels of enzymes in each enterocyte compartment included in the program. Scaling of gut expression levels with respect to whole liver enzyme content is used for the default expression levels of all of the common highly expressed CYP and UDP Glucuronosyltransferase (UGT) enzymes in the gut. The accuracy of GastroPlus™ to estimate drug concentrations in the intestinal enterocytes and the ability to accurately reproduce nonlinear dose dependence for substrates of CYP enzymes using only *in vitro* metabolic data has been demonstrated in the literature for UK-343,664, a P-gp and CYP3A4 substrate. This example represented an example of conducting such a simulation using mechanistic oral absorption compartmental absorption and transit models (Abuasal et al., 2012). GastroPlus™ has also been used to accurately model the nonlinear dose dependence for substrates of ATP-binding cassette efflux transporters and substrates for both CYP enzymes and efflux transport (Abuasal et al., 2012; Tubic et al., 2006).

GastroPlus™ has capabilities which allows for input into projects from discovery pharmacokinetics through clinical development. For example, it has been implemented in *in vitro–in vivo* extrapolations in animals and humans (e.g. De Buck et al., 2007), clinical formulation development and implementation of quality by design (e.g. Crison et al., 2012) and generation of IVIVC (e.g. Mirza et al., 2013).

Recently, GastroPlus™ was used by GlaxSmithKline (GSK) in the evaluation of different formulations of a weak acid compound developed as a salt. Whilst the current clinical formulation contained the sodium salt (Formulation A), the Project Team wanted to investigate the behavior of a formulation containing the less soluble free acid in either a nanomilled (Formulation B) or micronised (Formulation C) form. Following investigation in clinical study, whilst exposure was only slightly reduced for Formulation B, exposure was significantly lower for Formulation C compared to the current formulation.

GastroPlus™ was therefore used to investigate whether it was possible to obtain similar exposure levels to Formulation A using formulation C by either increasing the dose slightly or using a nanomilled formulation. The input parameters to the GastroPlus™ model were the measured solubility in Britton–Robinson buffers at pH 2, 4, 6, 8, 10 and 12, solubility in Simulated Gastric Fluid sine pepsin SGF_{sp}, FaSSIF and FeSSIF buffers, permeability in MDCK cells, and plasma protein binding. The human liver clearance was predicted from *in vitro–in vivo* Extrapolation. The tissue:plasma partition coefficients (K_{ps}) were predicted using the default Rodgers-Single (Lukacova) K_p method (Lukacova et al., 2008). Formulation-specific input parameters were the particle size distribution, the enhancement in solubility for the salt (Formulation A), and the enhancement in solubility due to the nanoparticle effect (Formulation B).

The GastroPlus™ model predicted that it would not be possible to get equivalent exposure to Formulation A with Formulation C at a modest increase in dose. The model also showed that the pharmacokinetics of Formulation B would be insensitive to changes in particle size in the nm range. This study, in which a mechanistic model of formulations is constructed to understand the impact of change is in alignment with the U.S. Food and Drug Administration (FDA) Quality-by-design (QbD) initiative (Yu, 2008).

2.3. PK-Sim®

PK-Sim® is a whole-body PBPK model distributed by Bayer Technology Services GmbH (Leverkusen, Germany). Currently, version 5 is available.

PK-Sim® 5 allows quantitative pharmacokinetic predictions not only in humans, but also in preclinical mammals like dogs, mice, minipigs, monkeys, and rats. Whilst mean population values of the anatomy and physiology for each species are available in the software, these values can be altered by the user (PK-Sim®, 2012).

The software contains detailed information of the age, gender, and race-related physiological parameters of various human study populations including black, white and Mexican Americans (NHANES, 1997), Europeans (Valentin, 2002); and Asians (Tanaka and Kawamura, 1996). Additionally, the different populations can be further modified, thereby enabling simulations to be conducted in special patient populations including obese and the renally impaired, in addition to children and the elderly. Using the Population Module, simulations can be performed in a virtual population with up to 1000 individuals (each having different anatomical and physiological properties).

As shown in Fig. 5, the structure of PK-Sim® 5 is based on compartmental models. In the current version, the model structure of

the GI tract consists of 12 segments (representing the stomach, duodenum, upper and lower jejunum and ileum, respectively, caecum, ascending, transverse, descending, and sigmoid colon, and rectum). Each of the different intestinal segments contains an additional mucosal segment (Thelen et al., 2011, 2012). The detailed description of the gut wall (including the mucosa) enables the implementation of various processes that take place in the gut wall, such as first pass metabolism, active transport mechanisms, and additionally encompasses drug–drug and drug–protein interactions. However, these reactions are not limited to the GI compartments and may be implemented in any relevant organ or tissue (PK-Sim®, 2012).

In PK-Sim® 5, all organs are presented as compartments and each compartment is subdivided into a vascular and an extravascular space. The vascular space is then further subdivided into plasma and red blood cells, whilst the extravascular space is further subdivided into the interstitium and the cellular space. The fractions of lipid, protein, and water in each organ and sub-compartment are embedded in the software, enabling the calculation of tissue–plasma-partition coefficients using five different approaches (PK-Sim® standard (PK-Sim®, 2012), Rodgers and Rowland (Rodgers et al., 2005a,b; Rodgers and Rowland, 2006, 2007), Schmitt (Schmitt, 2008), Poulin and Theil (Poulin et al., 2001; Poulin and Theil, 2000, 2002a,b), and Berezhkovskiy (Berezhkovskiy, 2004a,b)). Differential mass-balance-equations not only describe the transport of the drug from one sub-compartment to the other sub-compartment but also describe the transport from one organ to another.

For drugs applied via the oral route, the underlying assumption is that the drug needs to dissolve during its passage through the GI tract before being absorbed. PK-Sim® 5 allows the calculation of the pH-dependent solubility of ionizable compounds using the Henderson–Hasselbalch equation. To describe the solubility of the drug, the software allows the input of one solubility value, either in a blank buffer or in biorelevant media (e.g. FaSSIF or FeSSIF). However, the current version of the software does not support an *a priori* calculation of the drug's bile dependent solubility.

To describe the GI dissolution characteristics of an oral formulation, seven different input functions can be selected. These include the situation for an oral solution, user-defined dissolution which enables a direct upload of dissolution data from an Excel file, Weibull kinetics, a "lint 80" dissolution function (which describes linear dissolution until 80% of the drug is dissolved, then extrapolation to 100% dissolution), particle-size dependent dissolution based on Noyes–Whitney kinetics, zero order, and first order dissolution kinetics.

Neither a supersaturation ratio nor precipitation kinetics can be used to describe GI supersaturation or precipitation in PK-Sim® 5. Instead, precipitation is accommodated in the simulation by selection of either the "dissolved" or the "particle dissolution" function. In this case, the drug is allowed to precipitate according to its pK_a, its pH-dependent solubility profile, and the pH gradient along the GI tract in a kind of "reverse dissolution" step. Further, it is possible to take into account the possibility of the precipitate going back into solution (PK-Sim®, 2012).

To describe the permeability of the drug across the gut wall, experimentally determined values from Caco 2 or PAMPA assays can be used directly in the simulations. However, if these data are not available, the transcellular intestinal permeability can be calculated using the basic physicochemical properties of the drug, including lipophilicity, the effective molecular weight, and the pK_a (PK-Sim®, 2012). The software is able to accommodate situations in which the drug is a substrate for active transport mechanisms, active efflux, influx, and Pgp-like transport mechanisms.

PK-Sim® 5 describes first pass metabolism using various kinetic models, such as Michaelis–Menten or first order kinetics. For this purpose, the corresponding enzyme abundance for each organ (such as the liver or the various GI compartments) can be incorporated into the software. Age-dependent ontogeny for a broad variety of CYP and UGT enzymes are taken into consideration in PK-Sim® 5. Additionally, specific drug–protein- and drug–drug-interactions can be defined at the enzyme level. Scaling from *in vitro* to *in vivo* clearance is also possible (PK-Sim®, 2012).

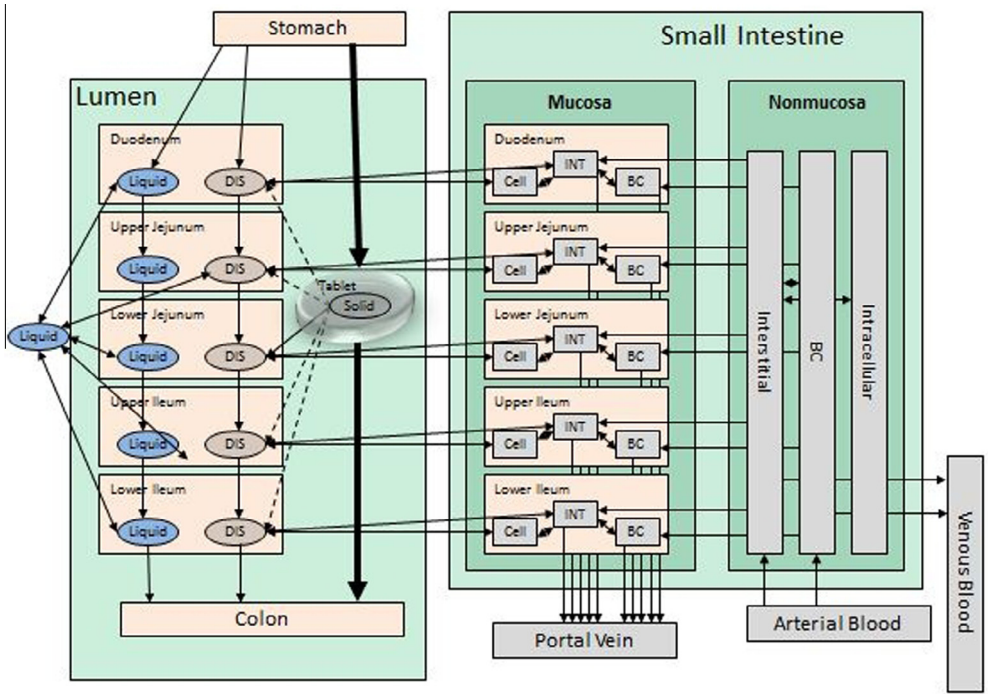

Fig. 5. Structure of the absorption model used in PK-Sim® (Version 5 and upwards). The model also includes the large intestine (not shown in this figure) which has a similar structure to the small intestine compartment (reproduced with permission from Thelen et al. (2012)).

An additional feature of PK-Sim® is that it can be combined with the MoBi® software (Bayer Technology Services GmbH, Leverkusen, Germany) which not only enables a completely new PBPK model to be built from scratch but also allows modifications of existing PK-Sim® models.

With PK-Sim®, the description of the anatomy and physiology for different species, enables, together with the MoBi® software, a detailed description of the events taking place during the absorption process and also immediately post absorption. Further, the application of the software is not just limited to pharmacokinetic simulations, but also enables the link between pharmacokinetics and pharmacodynamics to be examined. A search of the literature reveals that PK-Sim® is mostly used for describing highly complex pre- and post-absorptive processes, such as metabolism, mass-balance, and pharmacogenomics in different CYP phenotypes or population subgroups (Dickschen et al., 2012; Eissing et al., 2011b; Willmann et al., 2009a), the influence of drugs on the pharmacokinetics and pharmacodynamics of hormonal systems (Claassen et al., 2013; Eissing et al., 2011a) and pharmacokinetic scaling approaches (Strougo et al., 2012; Weber et al., 2012). In contrast, there are fewer publications that deal with describing drug absorption in humans and preclinical mammals (Thelen et al., 2010; Willmann et al., 2007, 2009b, 2003, 2010).

Recently, PK-Sim® was used to investigate the lack of dose-linearity in nifedipine pharmacokinetics (Thelen et al., 2010). *In vivo* data following oral administration of a soft gelatine capsule containing 5 mg, 10 mg, or 20 mg nifedipine in an IR format showed a dose-linear increase of the AUC. In contrast, linearity was not observed for C_{max} following administration, with sub-proportional increases at the 20 mg dose (Raemsch and Sommer, 1983). To estimate the factors that led to the marked reduction of C_{max} values in the absorption of nifedipine from the 20 mg dose, biorelevant dissolution tests in FaSSGF were performed with both 10 mg and 20 mg nifedipine. In the dissolution tests, precipitation of nifedipine was observed for the 20 mg formulation, but not for the 10 mg formulation. To describe the intralumenal dissolution of the drug, a Weibull function was fitted to the *in vitro* dissolution profiles of both formulations (10 mg and 20 mg) and implemented in the software. As nifedipine is known to exhibit significant first pass metabolism via CYP 3A enzymes, Michaelis–Menten kinetics were used to accommodate both small intestinal and hepatic CYP-mediated metabolism.

The simulation results showed that nifedipine plasma profiles could be simulated well by combining the *in vitro* dissolution test results with the PBPK software, and it could be demonstrated that the onset in nifedipine absorption following administration of 20 mg nifedipine was limited by gastric drug precipitation. The software, combined with *in vitro* dissolution tests, thus served as an important tool for investigating the intralumenal dissolution behavior of this poorly soluble drug, Additionally, this could only be achieved using a quantitative description of the gut wall metabolism of nifedipine (Thelen et al., 2010).

2.4. Other in silico tools

2.4.1. MATLAB®

Increased availability of commercial software platforms facilitates wider use of PBPK modeling as a 'learn and confirm' tool at different stages of drug development (Jones et al., 2011b; Parrott and Lave, 2008; Rostami-Hodjegan, 2012; Rowland et al., 2011; Sinha et al., 2012). In addition to commercial packages, a number of studies have reported the application of in-house, user customized models built in either MATLAB®, Berkeley Madonna, MoBi® or STELLA® (Bouzom et al., 2012; Gertz et al., 2013, 2011; Jones et al., 2012; Kambayashi and Dressman, 2013). These customized models provide a certain flexibility and can be extended/used for multiple purposes beyond generic PBPK, e.g., PBPK-PD (Claassen et al., 2013), to account for dissolution and precipitation kinetics (Kambayashi and Dressman, 2013) or modeling of *in vitro* transporter kinetics and other cellular process (Korzekwa et al., 2012; Menochet et al., 2012).

An example of the implementation of the mechanistic intestinal absorption model in MATLAB® has been reported recently for CYP3A substrates with high intestinal extraction (Gertz et al., 2011). The intestinal model within the whole body PBPK framework followed the principles of compartmental absorption and transit model (Yu and Amidon, 1999). For all the drugs investigated, absorption was considered to occur from the small intestinal compartments, with the exception of saquinavir, where colonic absorption was also incorporated, in agreement with previous reports (Agoram et al., 2001). The intestinal model allowed description of the changes of drug amount in the intestinal lumen corresponding to different intestinal segments of duodenum, jejunum and ileum and accounted for both undissolved and dissolved drug. Inclusion of the heterogeneous expression levels of CYP3A and efflux transporters along the small intestine allowed the prediction of enterocytic drug concentration in different intestinal segments and assessment of any potential nonlinearity/saturation of the intestinal first-pass. The latter has been implicated in the under-prediction of intestinal availability (F_G) observed with the use of minimal models as the Q_{Gut} model (Gertz et al., 2010, 2011; Yang et al., 2007). In addition to F_G predictions, this custom built PBPK model in MATLAB® allowed the assessment of apparent i.v. and oral clearance, with the majority of the drugs predicted within 3-fold of the observed data.

In another study, a cyclosporine PBPK model constructed in MATLAB® was applied to predict the effect of different formulations (Neoral® and Sandimmune®) on the magnitude of the inhibition of CYP3A4 metabolism and efflux via P-gp in different intestinal segments, together with the impact on hepatic counterparts. Due to slower absorption of cyclosporine from Sandimmune® formulation, the predicted reduction of transporter and enzyme activity was lower and the effect was more protracted in comparison to Neoral® (microemulsion) (Gertz et al., 2013).

Depending on the specific questions that need to be addressed, application of a reduced or semi-PBPK model can in some instances be more advantageous than a whole body model. This is in particular the case when the focus of the analysis is on a specific organ, either due to drug–drug interaction or absorption concerns (e.g., liver and intestine) or safety issues (e.g., muscle in the case of statins). By doing so, the number of tissue compartments is minimized and tissues of less relevance/interest can be lumped together; this is often performed by grouping together tissues which show comparable perfusion and distribution equilibrium characteristics (rapid or slow) (Nestorov et al., 1998). In some instances, the model can be reduced to either just a central compartment or with a consideration of an additional peripheral compartment, while keeping a mechanistic description of metabolism/transporter processes in the organs of interest, e.g., intestine (Ito et al., 2003; Quinney et al., 2010; Zhang et al., 2009). These reduced models are more amenable to parameter optimization whilst still retaining physiological relevance and extrapolative power. The reduced PBPK models have been used to describe nonlinear drug disposition (e.g., clarithromycin) (Quinney et al., 2010) and to predict the magnitude of intestinal interaction in addition to liver, as illustrated in the midazolam and diltiazem example (Zhang et al., 2009).

2.4.2. STELLA®

A simple integrated PBPK model into which a user can build functionality can be achieved through the use of the STELLA® model platform (Cognitus Ltd., North Yorkshire, UK). STELLA®

(Structural Thinking Experimental Learning Laboratory with Animation) is an icon-based model building and simulation tool that was first introduced in the 1980s. In contrast to commercially available PBPK models available, through the use of a graphical interface, the user constructs and runs the simulation by building a graphical representation of the model.

The utilization of STELLA® in PBPK modeling was first realized in the late 1980s (Grass and Morehead, 1989; Washington et al., 1990). In one early publication, STELLA® was used to examine the potential influence of CR formulations of moexipril on the potential effects on plasma concentrations (Grass and Morehead, 1989). This was achieved by the input of *in vitro* dissolution data into the model, Since then, the functionality of the models have become more elaborate and the STELLA® model has been used to not only simulate but also accurately predict human plasma profiles through the addition of using biorelevant *in vitro* dissolution data and, more recently, also *in vitro* precipitation kinetics (e.g. (Juenemann et al., 2011; Nicolaides et al., 2001; Shono et al., 2011; Wagner et al., 2012).

As an example, STELLA® software has been successfully used to accurately predict plasma profiles for different fenofibrate lipid-based formulations under fasting conditions in humans (Fei et al., 2013). For the simulations, *in vitro* data from dispersion/dissolution (of the dosage forms), precipitation as well as re-dissolution of the precipitate were taken into account in the model. Using the STELLA® model map shown in Fig. 6, the plasma profiles for the different formulations were in very good agreement with the *in vivo* plasma profiles for these formulations.

Whilst the STELLA® platform is very useful if the basis of data available is limited, it is essential that *in vivo* pharmacokinetic data, in particular the elimination rate constant, are available if the goal is to simulate/predict plasma profiles. Further, when several factors can directly influence the absorption characteristics including first-pass metabolism, interaction with P-gp and regional differences in absorption, all of these parameters would need to be included in the model. In such cases, the model map may become quite complicated to set up and it may be more relevant to perform these simulations in one of the commercially available software packages that have the various functionalities already incorporated in the model.

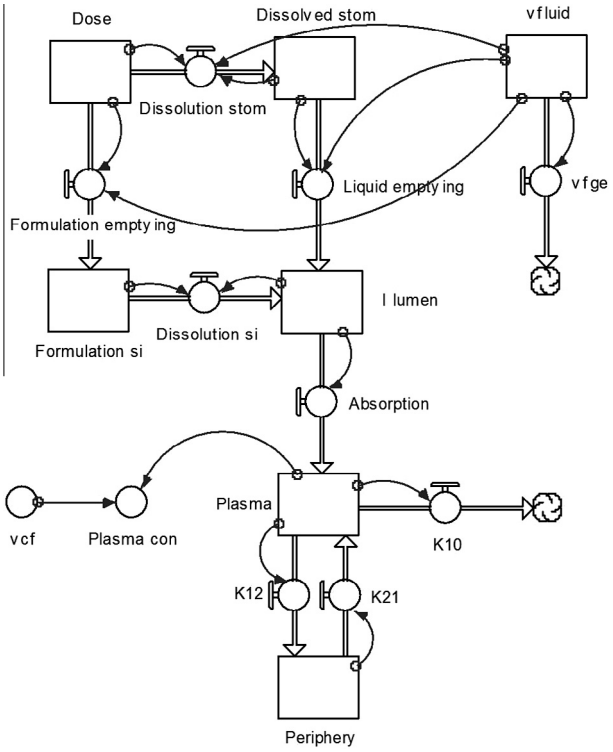

Fig. 6. Model map used in STELLA® to simulate plasma fenofibrate concentrations in plasma following oral lipid formulations (reproduced with permission from Fei et al. (2013)).

2.4.3. GI-Sim

The internal AstraZeneca absorption model GI-Sim deploys a compartmental physiological model for the GI tract in combination with up to three compartments to describe the plasma concentration–time profile. The human physiological model adopted in GI-Sim constitutes of nine GI compartments coupled in series; the stomach (1), the small intestine (2–7) and the colon (8–9) (Fig. 7) (Sjögren et al., 2013). The description of the underlying physiology has been reported previously (Yu and Amidon, 1998, 1999; Yu et al., 1996a). The physiological parameters for the different GI compartments were adopted from Heikkinen et al. (2012a).

In GI-Sim, undissolved particles and dissolved molecules flow from one intestinal compartment to the next. In contrast, the bile salt micelles present in the small intestine are modeled with a constant concentration and a calculated micellar volume fraction of 0.0002 (Persson et al., 2005b) in each small intestinal compartment. Each compartment is ideal, i.e. concentrations of dissolved and undissolved drug, pH, etc. are the same throughout the compartment, apart from a thin Aqueous Boundary Layer (ABL) at the intestinal wall. Each compartment has a specific biorelevant

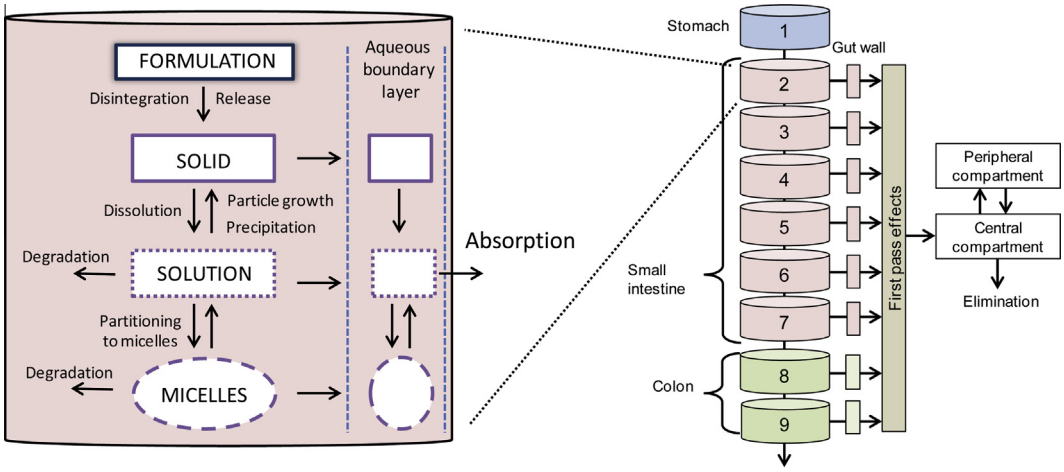

Fig. 7. A schematic view of the absorption model used in GI-Sim.

pH and as a consequence the solubility of an ionisable compound changes along the GI tract. Within a compartment, particles may either dissolve or grow and dissolved molecules may partition into the bile salt micelles or be transported across the intestinal membrane. The area available for absorption in each compartment is calculated, assuming an ideal tube, from the compartment volumes and a mean radius (weighted by the segments length) of 1.15 cm. An area amplification factor, which decreases in the distal compartments, is included to account for how folds, villi and microvilli structures affect the area available for absorption (Mudie et al., 2010; Willmann et al., 2004).

In GI-Sim, the pH-dependent solubility of a compound is described by the Henderson–Hasselbalch equation and dissolved uncharged molecules may also partition into the micelles in the small intestinal compartments (Sjögren et al., 2013). The rate of dissolution and crystalline nucleation is described by Fick's law together with the Nielsen stirring model while the rate of crystalline nucleation is modeled by a modified version of the crystalline nucleation theory (Lindfors et al., 2008; Nielsen, 1961).

The membrane transport process in GI-Sim is modeled as a serial diffusion through the ABL of thickness L, with permeability P_{ABL}, and a membrane, with the permeability P_m. Together they constitute a barrier to membrane transport and absorption with the total effective permeability P, described by:

$$\frac{1}{P} = \frac{1}{P_{ABL}} + \frac{1}{f_0 \cdot P_m} \quad (1)$$

where f_0 is the uncharged fraction. The human effective permeability input in GI-Sim is estimated from an established correlation between measured human effective permeability and apparent permeability from the Caco-2 model for a number of reference drugs. To account for differences in ABL thickness due to inadequate stirring *in vitro*, the correlation is based on P_m rather than P. Undissolved drug particles diffuse slower than free drug monomers across the ABL but contain many molecules and may therefore contribute substantially to the P_{ABL}. In GI-Sim, the diffusion coefficient of particles across the ABL is inversely proportional to the particle radius. The general effect of particles is that P_{ABL} increases with increasing concentration of particles. This effect will be especially important for small nanosized particles and contribute to the improved absorption provided by such formulations. GI-Sim also includes other functionalities such as luminal degradation, drug release profiles e.g. for CR formulations as well as physiology models for pre-clinical species. The contribution of gut wall and first-pass liver metabolism to BA is estimated as a combined first pass effect.

The accuracy of the absorption model in GI-Sim regarding prediction of f_{abs} and plasma exposure in humans has been evaluated by simulating the f_{abs} of twelve compounds reported to be incompletely absorbed in humans due to permeability, solubility and/or dissolution rate limited absorption (Sjögren et al., 2013). The overall predictive performance of GI-Sim was good as >95% of the predicted C_{max} and AUC were within a 2-fold deviation from the clinical observations and the predicted plasma AUC was within one standard deviation of the observed mean plasma AUC in 74% of the simulations (Fig. 8). GI-Sim also captured the trends in dose and particle size dependent absorption of the study drugs as exemplified by AZ2 and digoxin (Fig. 9). In addition, GI-Sim was also shown to be able to predict the increase in absorption and plasma exposure achieved with nano formulations. Based on the results, the performance of GI-Sim was shown to be suitable for early risk assessment as well as to guide decision making in pharmaceutical formulation development.

3. Current challenges in PBPK setups for GI absorption

3.1. Composition of luminal contents

From an oral drug release and absorption perspective, the most important features of the GI tract in humans are the stomach, small intestine and proximal large intestine. In addition, secretions from the various accessory organs (including the gall bladder and pancreas) that supply the small intestine play a significant role.

It is also important to consider that the GI tract is not a static environment. Rather, not only does the physiological state alter between fasting and fed conditions, but also as the drug/dosage formulation transits through the GI tract, the environment to which it is exposed is continuously changing. To complicate things even further, inter-individual variability in GI physiology is an additional aspect that needs to be considered in the PBPK model.

The most important parameter of the fasted state stomach is the acidic pH, which is typically below pH 2, but can range between 1 and 7.5 (Dressman et al., 1990; Lindahl et al., 1997). Following food intake, gastric pH almost instantaneously increases to between 4 and 7, depending on the nature of the meal (Apostolopoulos et al., 2006; Carver et al., 1999; Dressman et al., 1990; Kalantzi et al., 2006a). Gastric pH thereafter reduces to fasting levels within approximately 2–5 h after a solid meal (Dressman et al., 1990; Kalantzi et al., 2006a; Russell et al., 1993).

In the fasting stomach, the contents are typically hypo-osmotic and influenced by the volume of water administered. Another important parameter is surface tension, which even under fasting conditions is much lower than that of water (72 mN/m) and with food can be even lower, depending on the nature of the meal (Efentakis and Dressman, 1998). In the stomach there are also two main digestive enzymes that may be important in terms of drug delivery. Pepsin, a proteolytic enzyme, is able to catalyze the degradation of peptides and protein drugs. Also lipases, which are found in small quantities in the gastric fluid, are important for initiating

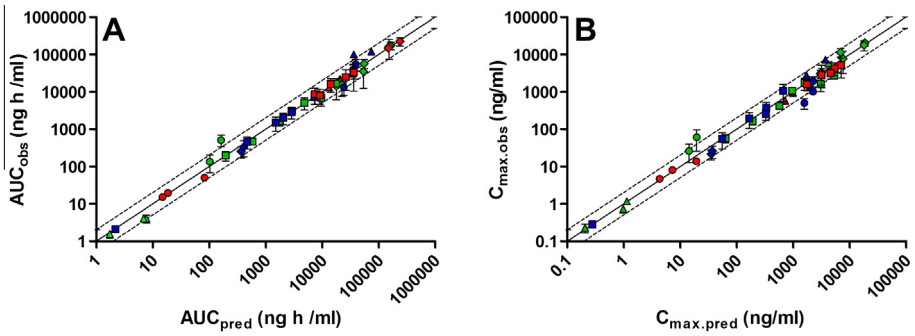

Fig. 8. Overview of the overall predictive performance of GI-Sim. The graphs depict the accuracy in predicted AUC (A) and C_{max} (B). Error bars in observed AUC and C_{max} represent standard deviation. Different drugs are indicated by color and shape, the solid line and the dotted lines represent the line of unity and a 2-fold difference, respectively.

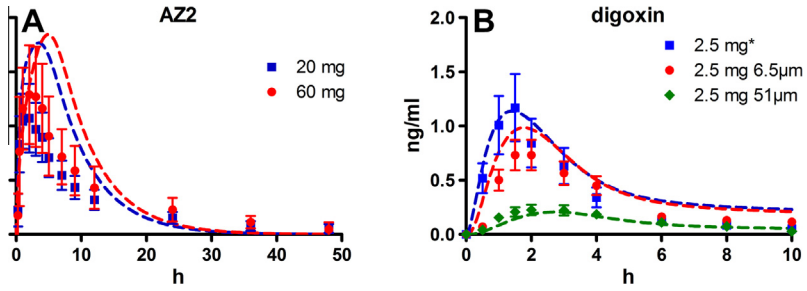

Fig. 9. Observed (symbols) and predicted (dotted lines) plasma concentration time profiles of AZ2 (dose dependent/solubility limited absorption) (A) and digoxin (particle size/dissolution limited absorption) (B). Administration of solutions are indicated by a star (*).

fat digestion in the stomach. As such they may also influence the behavior of lipid based formulations. (Porter et al., 2008).

The next main site is the small intestine, the pH of which is influenced by the pH of the gastric contents entering the small intestine and the buffering nature of the pancreatic secretions. Additionally, direct bicarbonate secretion contributes to the rise in pH along the small intestine towards the ileum. Under fasting conditions, the pH in the upper small intestine is highly variable with mean values of pH 6.5, but can range anywhere between as low as 2 just below the pylorus up to around 7 in the mid to distal duodenum. pH values in the upper small intestine can be influenced by the phase of the Myoelectric Motor Complex (MMC) (Woodtli and Owyang, 1995). Following food, the pH gradually decreases due to the emptying of acidic gastric contents and then returns to fasting state values following complete gastric emptying. By the mid to distal ileum, pH has increased to around 7.5–8.0 (Dressman et al., 1990), predominantly due to a combination of absorption of nutrients and direct, local bicarbonate secretion, leading to a lack of food effect on the pH value in the distal small intestine.

Through the action of bile acids, cholesterol and other lipids, the proximal small intestine contains a mixture of mixed micelles, liposomes and emulsion droplets. These aggregates can not only enhance the solubility of lipophilic drugs but can also interact with the dosage form or even with specific excipients contained within the formulation. Under fasting conditions, intestinal chyme contains bile acids in the 2–6 mM concentration range and low concentrations of phospholipids (0.19–0.26 mM). Under fed conditions, the concentrations of bile salts can increase to a mean value of around 15 mM, and approximately 3 mM for phospholipids (Kalantzi et al., 2006b; Persson et al., 2005a, 2006) (Bergström et al., 2013 in this issue of EJPS). Under both fasting and fed conditions, due to the constant presence of surface-active components, surface tension is low, approximately 30 mN/m (Persson et al., 2005a) and can thus exert a direct impact on drug wettability and hence drug dissolution.

The presence of food triggers the secretion of digestive enzymes from the pancreas into the small intestine. There are three major types of enzymes secreted for the digestion of carbohydrates (amylases), lipids (lipases) and protein (proteases). From a drug absorption perspective, lipases are the most relevant in terms of their ability to digest lipid formulations in addition to the proteases and their influence on the stability of peptide drugs.

In contrast to the proximal GI tract, the colonic fluids are less well characterized and this is reflected by the less detailed description of the distal regions of the GI environment in the PBPK models currently available. The main relevant activities important to drug dissolution and absorption are fermentation by the large bacterial population present and reabsorption of water and electrolytes. Due to the fermentation of food residues into short chain fatty acids (SCFA), the pH drops from the terminal ileum to ascending colon to approximately pH 5.5–6.0 (Diakidou et al., 2009; Nugent et al., 2001). Thereafter, following absorption of the SCFA's, pH increases in the distal colon to approximately 7 in the descending colon/rectum. Whilst the concentration of bile salts in the large intestine are relatively low compared to the small intestine, the surface tension of the colonic fluids remains quite low (approximately 40 mN/m) (Diakidou et al., 2009).

In addition, PBPK models need to consider the large number of metabolically active bacteria present in the terminal GI tract and their mechanism for degradation, which could also influence drug stability (McConnell et al., 2008; Sousa et al., 2008) and ultimately the fraction of dose absorbed.

3.2. Transit and hydrodynamics

The GI transit of drug substance in both solid and liquid forms (i.e. in solution or suspension) can be of great importance for the absorption of drugs due to the distinctly diverse physiochemical conditions in different regions of the GI tract (Varum et al., 2010). A complicating factor in describing these processes is that the GI distribution for solid particles and liquids are inherently different (Davis et al., 1986; Varum et al., 2010). Therefore, it is necessary to consider whether the drug remains within the formulation and hence is moving in the form of a solid particle, or if it is dissolved or suspended in the intestinal fluids. Whilst the majority of the PBPK models currently available (Agoram et al., 2001; Jamei et al., 2009b; Willmann et al., 2009b) recognize this general difference, most effort has been put into characterizing the GI distribution of dissolved/suspended drug, probably as a consequence of focusing on predicting the absorption of poorly soluble drugs and for these cases, transit has often been assumed similar for suspended and dissolved drug.

Under fasting conditions, the transit of solid formulations through the upper GI tract (stomach and small intestine) is primarily governed by the MMC (Coupe et al., 1991; Husebye, 1999). This results in a distinct cyclic pattern of electromechanical activity that triggers peristaltic waves that originate from the stomach and propagates through the small intestine. An MMC cycle consists of 4 distinct phases, reoccurring every 1.5–2 h in the fasting state (Dooley et al., 1992; Sarna, 1985). Postprandially, MMCs disappear, to be replaced by a digestive motor activity characterized by regular mixing and propelling movements that optimize nutrient absorption (De Wever et al., 1978).

Larger solid objects such as capsules or single unit tablets have been demonstrated to have significantly different GI transit patterns (with respect to gastric emptying and colon transit times) compared to solutions or small solid units (e.g. pellets) (Abrahamsson et al., 1996; Davis et al., 1986; Varum et al., 2010). For gastric emptying, there is a dependency on the size of the formulation and this is most obvious when the dose is administered together with food (Davis et al., 1986; Khosla and Davis, 1990). Smaller units and

dissolved drug are typically emptied significantly faster than larger units. This is in line with the stomach's functionality of grinding the solids down to a manageable size before emptying into the duodenum. For large non-disintegrating capsules given in combination with heavy meals, gastric emptying has been reported as late as 10 h post dosing (Davis et al., 1986). When administered in the fasting state, gastric emptying is generally fast and for most pharmaceutical granules and pellets with diameter less than 2 mm are typically emptied within a less than 1 h (Davis et al., 1986).

Also movement within the stomach can play a significant role especially on the absorption characteristics from MR formulations. Following dosing in the fed state, where MR formulations may remain longer in the stomach, transit between the proximal stomach (fundus) and the distal stomach (antrum) can have an impact on the release and subsequent absorption characteristics (Weitschies et al., 2005). Apart from the fact that drug substance released in the proximal stomach is emptied from the stomach more slowly than drug release in the distal stomach, there can also be differences in the dissolution rate due to lower mechanical stresses and higher postprandial pH in the proximal compared to the distal stomach (Bergstrand et al., 2012a,b, 2009). Therefore, the inclusion of within-stomach movement in PBPK models might be an important consideration for the prediction, especially for MR formulations.

Small intestinal transit is less dependent on the size of the formulation and concomitant food intake. If anything, solid particles appear to transit slightly faster than the liquid content (Davis et al., 1986; Yuen, 2010). Fluid volumes along the GI tract have been measured by Schiller and co-workers using water-sensitive Magnetic Resonance Imaging (MRI) in 12 healthy volunteers (6 female) (Schiller et al., 2005). The results from this study provided evidence that GI fluid is not continuously available throughout the GI tract but is found in clusters. Therefore, assuming fixed volumes for the individual small intestinal compartments, as in the case in the generic PBPK models, may lead to incorrect predictions.

It would also be important for PBPK models to consider the extent to which the volume of fluid within the gut lumen (V_{lumen}) changes with time as a result of fluid intake, secretion and reabsorption, as this could have a significant effect on the dissolution of a drug and hence the concentration presented to enzymes and transporters within the enterocyte.

GI transit is a heterogeneous process for a solid dosage form, which sometimes moves quickly, sometimes slowly, experiencing fluids of varying composition, and being subject to varying peristaltic pressures (Weitschies et al., 2010). A typical small intestinal transit time for solid dosage forms in healthy subjects is reported to range between 2 and 4 h. However, there is a considerable between and within subject variability, with values ranging between 0.5 and 9.5 h. It should also be recognized that a considerable part of the small intestinal residence time is spent at rest rather than the object moving continuously through this region (Weitschies et al., 2010).

Assuming that fluid movement along the GI tract is consistent with the rate of gastric emptying and small intestine transit, ordinary differential equations can be used to simulate the change in fluid volume in the stomach and each intestinal segment. The gastric emptying and small intestine transit time, regional fluid secretion and reabsorption rates and the baseline fluid volumes are all affecting the regional fluid volume. As expected, the between and within subject variability of any of these processes can contribute to the observed systemic variability.

Before entering the colon, solid dosage forms typically stagnate in the caecum for a variable period of time. Food intake stimulates emptying of cecum into the colon, a mechanism that is known as the gastro-ileocecal reflex (Schiller et al., 2005; Shafik et al., 2002). Further, transit of dosage forms through the colon is highly variable and appears to occur during periods of relatively fast movement followed by long periods of rest (Adkin et al., 1993). The movement periods may be stimulated by food intake but this is not always the case (Weitschies et al., 2010). The size of the particles has been shown to influence the movement through colon with small solid pellet particles moving slower than larger single unit formulations (Abrahamsson et al., 1996; Adkin et al., 1993; Davis et al., 1984). At present, these aspects of GI transit have not been characterized in enough detail to be represented well in the PBPK models.

The between and within subject variability is large with respect to almost all aspects of GI transit, even for homogeneous healthy populations studied under highly controlled conditions. This variability can for many substances and formulations also propagate into large variability in systemic exposure and treatment success. Taking into account the fact that disease and pharmacological treatments can also alter GI transit (Varum et al., 2010) and the fact that feeding habits generally vary more in daily life than in a controlled clinical study setting, the true expected population variability in clinical practice is likely to be substantially larger.

Another important consideration is the influence of circadian rhythm on GI transit. Whilst few studies have been undertaken to examine this more closely, it is known that a relatively longer gastric residence time has been described for tablets administered at night time compared to day time (Coupe et al., 1992a,b) and that the total residence time in the colon is likely prolonged due to patterns of bowel movement (Sathyan et al., 2000). It is hoped that further studies evaluating circadian rhythm on GI transit will be undertaken so that this can be appropriately incorporated into PBPK models and thereby enable simulations to be achieved for the purpose of predicting the influence of dosing regimens other than the typical morning dosing schedule.

PBPK models are also serving an important function to carry out predictions for pediatric populations (Barrett et al., 2012; Björkman, 2005). Similar to the lack of knowledge as to how GI transit differs in elderly and diseased subjects, little is known about potential differences in the pediatric population. The current knowledge base has been summarized in two recent review articles (Bowles et al., 2010; Kaye, 2011). Incorporation of this information in PBPK models could aid in development of formulations better tailored to special needs in the pediatric population.

3.3. Permeability, including transporters

Physiologically-based intestinal models contain enterocyte compartments corresponding to a particular intestinal segment; the entry of the drug into enterocyte is defined by its effective permeability (Lennernas, 2007) and a radius of that intestinal segment (Yu et al., 1996b). Therefore, drug permeability, together with *in vitro* metabolic clearance data, represents one of the key input parameters in physiologically-based intestinal models (Gertz et al., 2011; Heikkinen et al., 2012a; Jamei et al., 2009b; Pang and Chow, 2012; Yang et al., 2007). With these methods, permeability in the lower GI tract can be estimated based on surface area differences for transcellular and passive absorption, but for paracellular and active uptake mechanisms, estimations of permeability in these regions remains a challenge.

In vitro apparent permeability (P_{app}) data may be generated in either non-cell based (PAMPA) or cell based systems (MDCK, Caco-2, LLC-PK1). Transfected cell lines used for the assessment of passive permeability should have low expression levels of endogenous transporters (Hilgendorf et al., 2007) or should be used in the presence of a an inhibitor of active processes e.g., P-gp inhibitor GF120918 (Thiel-Demby et al., 2009), to allow unbiased estimation of passive permeability. *In vitro* permeability assay

development, impact of the cell line, surfactants and time points have been discussed in detail elsewhere (Avdeef et al., 2007; Chiu et al., 2003; Matsson et al., 2005; Polli et al., 2001; Thiel-Demby et al., 2009). Use of either isotonic or gradient pH (6.5 and 7.4) for the permeability assays will affect the fraction ionized and potentially bias P_{app} (A–B) estimates depending on the physicochemical properties of the compound investigated (Neuhoff et al., 2003). Assessment is generally performed at a single concentration below the anticipated luminal drug concentration which may result in over-estimation of the contribution of active efflux processes.

Alternatively, effective permeability (P_{eff}) can be obtained from *in vivo* measurements by a perfusion system (Loc-I-Gut) in the upper jejunum at a pH 6.5 and at therapeutic dose of the drug. This method provides a net estimate of paracellular, passive transcellular diffusion and transporter mediated uptake or efflux; however, availability of such data is limited to approximately 35 compounds reported by Lennernas and colleagues (Knutson et al., 2009; Lennernas, 2007; Lennernas et al., 1993). A number of studies have reported the correlation between P_{app} in either Caco-2 or MDCK and P_{eff} values obtained in upper jejunum (Gertz et al., 2010; Sun et al., 2002); these literature or in-house generated regression analyses for the same dataset can subsequently be used to predict the P_{eff} for any new developing drug. It is important to note that available P_{eff} values are associated with large inter-individual variability (on average 70%) which in some cases exceeds 100% (e.g., amoxicillin) (Chiu et al., 2003; Winiwarter et al., 1998). The use of these empirical regression equations for drugs with $P_{app} < 10$ nm/s may be problematic considering the large scatter of the data in this range (Gertz et al., 2010; Sun et al., 2002).

Drug permeability is often incorporated in the intestinal models as a hybrid parameter together with enterocytic blood flow, as in the Q_{Gut} model (Gertz et al., 2010; Yang et al., 2007), enabling description of either permeability or perfusion rate limited scenarios. Predictive utility of permeability data obtained in different cell lines has recently been assessed for a diverse set of 25 CYP3A substrates. Differences in the F_G prediction success using the Q_{Gut} model were minor regardless of the cellular system used (MDCK and Caco-2), with high degree of prediction accuracy for drugs with *in vivo* $F_G > 0.5$. In contrast, imprecision was increased for a subset of 11 drugs with high intestinal extraction (Gertz et al., 2010). In the same study, use of permeability data predicted from polar surface area and hydrogen bonding potential resulted in the most biased F_G predictions and significant under-prediction trend. This *in silico* approach is not recommended for drugs with high polar surface area (e.g., saquinavir), as the validity of the existing regression equation (Winiwarter et al., 1998) was not established for drugs within that chemical space.

The increase in the complexity of intestinal models over the years is associated with increased availability of some of the system parameters, e.g., heterogeneous expression levels of CYP3A and efflux transporters along the small intestine (Berggren et al., 2007; Harwood et al., 2012; Mouly and Paine, 2003; Paine et al., 2006, 1997; Tucker et al., 2012). However, in contrast to some metabolic enzymes, absolute abundance data for many intestinal transporters, regional differences in these estimates and inter-individual variability are generally lacking. In addition, information on the correlation between expression of different transporters (e.g., P-gp vs. BCRP) or transporter-enzyme expression (e.g., BCRP vs. CYP3A4) in the same individuals is limited for intestine.

Kinetic characterization of intestinal transporters and metabolic enzymes over the range of drug concentrations is the preferable way of obtaining *in vitro* input parameters for the mechanistic intestinal models, allowing the model to account for any potential saturation of the protein of interest. Lack of such extensive *in vitro* kinetic data for the intestinal efflux transporters (together with transporter abundance and inconsistency in scaling), can be a limiting factor for the mechanistic prediction of oral drug absorption. Recently, compartmental modeling approaches have been discussed for the *in vitro* determination of kinetic parameters for efflux transporters, highlighting the limitation of commonly applied enzyme kinetic principles directly to monolayer flux data, as this leads to incorrect parameter estimates (Kalvass and Pollack, 2007; Korzekwa et al., 2012; Zamek-Gliszczynski et al., 2013). The analysis has also emphasized the need to consider intracellular rather than the media drug concentration as relevant for the interaction with the efflux transporters; however, such mechanistic transporter kinetic data are currently not available for a large number of drugs.

3.4. Intestinal wall metabolism

A recent analysis of the relative contributions of the fraction absorbed (F_a), F_G and the fraction escaping hepatic elimination (F_H) on BA on 309 drugs studied in human has indicated that for 30% of the compounds F_G was <0.8, highlighting the importance of incorporating intestinal metabolism in both BA and dose predictions in drug discovery and development (Varma et al., 2010). However, this is often not the case and the lack of consideration of extrahepatic metabolism or incorrect assumption of its minimal relevance may result in under-prediction of oral clearance (Poulin et al., 2011). The estimation of F_G is confounded by the difficulties in defining the exact contribution of the intestine from conventional i.v/oral dosing strategies either in human (Galetin et al., 2010) or *in vivo* animal models without employing more labor intensive cannulation based studies (Matsuda et al., 2012; Quinney et al., 2008), and a comprehensive knowledge of species differences in intestinal metabolism.

Enterocytes contain a range of CYP (de Waziers et al., 1990; Paine et al., 2006), and conjugation enzymes (e.g., UGTs, sulfotransferases) (Riches et al., 2009; Strassburg et al., 2000). Intestinal enzymes show a large intra- as well as inter-individual variability (Paine et al., 1997; Thummel et al., 1996) and differential expression along the length of the small intestine, with the highest level in the proximal regions (Galetin et al., 2010; Paine et al., 2006); comparable distribution patterns in both expression and activity along the human intestine have been reported for CYP and UGTs (Paine et al., 1997; Strassburg et al., 2000; Tukey and Strassburg, 2001; Zhang et al., 1999). Zonal enzyme expression in the intestine reflects the need to compartmentalize the intestine within the PBPK models in order to reflect these regional differences in metabolism (Gertz et al., 2011; Jamei et al., 2009b; Pang, 2003). This is of particular relevance for modeling of metabolism of compounds administered as Extended Release (ER) formulation designed to escape extensive first-pass metabolism by dissolution in regions of low metabolic capacity (Paine et al., 1997), or drugs for which solubility may be altered by changes in GI pH or bile salt secretions (Dressman and Reppas, 2000).

In vitro metabolism data may be obtained using intestinal microsomes and corresponding cofactors for CYP and UGT metabolism (Cubitt et al., 2009; Gertz et al., 2010) or cytosol (Cubitt et al., 2011) to account for pathways such as sulfation. Caution should be applied when utilizing microsomal data from samples obtained by microsomal scraping due to reported reduced enzyme activity and protein yield in comparison to enterocyte elution (Galetin and Houston, 2006). Alternatively, hepatic microsomes can be used for the initial assessment of intestinal CL_{int} following the normalization for enzyme abundance, as illustrated in the case of CYP3A substrates where CYP3A4 metabolic activities were comparable between the liver and intestine once expressed per pmol CYP3A (Galetin and Houston, 2006; Gertz et al., 2010; von Richter et al., 2004). This approach has limitations if there are uncertainties

about the main enzymatic route of elimination and in the case of any potential species/organ differences in metabolic pathways. The latter is highlighted in the example of raloxifene metabolism, where its 6β-glucuronide is a primary metabolite in rat liver and intestinal microsomes and also in human liver. In contrast, 4′β-glucuronide is the major metabolite in the human small intestine, formed by UGT1A10 which is selectively expressed in the human intestine. Differential enzyme expression and metabolic activity result in substantial species differences and much higher BA in rat (39%) relative to human (2%) (Jeong et al., 2005).

Extrapolation of intestinal *in vitro* metabolism data within PBPK models is to some extent limited by the lack of robust scaling factors for intestinal microsomal and cytosolic scaling factors. These are generally based on limited datasets in contrast to liver (Barter et al., 2007) and from samples prepared by mucosal scraping, which can bias the estimate for reasons stated above (Cubitt et al., 2009, 2011; Paine et al., 2006). Regional differences have been reported for the intestinal microsomal recovery (14.5–23.5 mg protein/g mucosa for duodenum and ileum, respectively, (Cubitt et al., 2009; Paine et al., 1997). In contrast, a single value for the whole intestine has been reported for scaling of intestinal cytosolic metabolic data (Cubitt et al., 2011), which may bias the assessment of the contribution of intestinal sulfation relative to hepatic. PBPK modeling of intestinal conjugation metabolic data in particular is confounded by the lack of absolute enzyme abundance data and regional differences in these estimates. Protein expression (both for enzymes and transporters) and data on inter-individual variability from large cohort of individuals with appropriate covariate analysis are required to increase the confidence in PBPK modeling of intestinal metabolism. Emerging LC-MS/MS based techniques are aiming to bridge these gaps in both human and preclinical species (Heikkinen et al., 2012b; Ohtsuki et al., 2012) and drive more physiologically relevant intestinal PBPK models.

3.5. Integration of results from in vitro models with PBPK modeling

3.5.1. How to best handle gut solubility in Computational Oral Absorption Simulation

Drug formulation release/disintegration and dissolution are the first steps to achieving BA for orally administered drugs. In turn, the physicochemical properties of a drug determine the inputs to theoretical models of formulation-specific release and dissolution. Finally, the interaction of those properties and theoretical models with a mechanistic oral absorption model of gut physiology results in simulations that explain experimental observations and are predictive of preclinical and human BA and pharmacokinetics (Bungay et al., 2011; Jones et al., 2011a,b; Watson et al., 2011; Yamazaki et al., 2011). This section will focus on the best methods to estimate *in vivo* solubility used in Computational Oral Absorption Simulation (COAS) (Sugano, 2009b).

3.5.1.1. Solubility in water and biorelevant media. Solubility is the primary, but not the only variable that affects dissolution and precipitation. Solubility can be defined and measured by many different methods, resulting in simulations with a wide range of variability (Avdeef, 2007). Strong discipline is required to acquire and utilize experimental inputs that have the highest "biorelevance" and avoid the temptation to deviate from accurate physiological parameters in order to "fit" experimental data. After all, the objective in all simulation modeling is not to make the smooth simulation line go through the experimental data points; rather, it is to initiate a simulation study with the most accurate physicochemical properties and physiological models and use the outcome to drive the development program.

Ultimately, knowledge of solubility as a function of *in vivo* pH is required in COAS to obtain the most accurate results. However, solubility in water (native solubility without buffers or surfactants) and the resulting value of "native pH" at equilibrium are the most important experimental inputs to GI absorption simulation for a variety of reasons. First, a single value of aqueous solubility is an inexpensive measurement and can be used in conjunction with Henderson–Hasselbalch theory to validate other experimental observations such as pK_a values and the shape of the aqueous solubility vs. pH profile (Avdeef et al., 2000; Bergstrom et al., 2004). Second, the aqueous solubility serves as a starting point for estimating the change in solubility due to the influence of bile salts *in vivo* (Bakatselou et al., 1991; Glomme et al., 2007; Mithani et al., 1996). Since bile salt concentration *in vivo* decreases going down the small intestine and is different between species and in the pre- and post-prandial state, a flexible, accurate method of calculating the dynamic changes in true *in vivo* solubility is essential to accurately predict results from COAS (Dressman et al., 2007; Jantratid et al., 2008; Sugano et al., 2007).

3.5.1.2. Methods to account for the influence of bile salts on in vivo solubility. Two methods can be used to account for the influence of bile salts on *in vivo* solubility, depending on whether or not the experimental data for solubility in biorelevant media is available. In the absence of experimental data, a theoretical approach can be utilized. One of the earliest theoretical treatments was based on a simple linear function of the octanol/water partition coefficient (Mithani et al., 1996). Mithani et al. measured the solubility of six neutral steroidal molecules in the presence of various concentrations of sodium taurocholate. Eq. (2) describes the *in vivo* solubility, $C_{s,bile}$, which is calculated from the aqueous solubility corrected for the solubilization ratio (SR) and the bile salt concentration in a given region of the small intestine accounting for fasted or fed conditions.

$$C_{s,bile} = C_{s,aq} + Sc_{aq} \times SR \times M_w \times [bile] \quad (2)$$

where $C_{s,aq}$ is the aqueous solubility, $C_{s,bile}$ is the solubility in the presence of bile salts at concentration [*bile*], S_{caq} is the aqueous solubilization capacity calculated as the ratio of moles of drug to moles of water at a concentration equal to aqueous solubility, Mw is the drug molecular weight, and SR is the bile salt solubilization ratio.

Eq. (3) is the theoretical relationship published by Mithani for changes in the solubilization ratio as a function of log*P*.

$$\log SR = 2.23 + 0.61 \times \log P \quad (3)$$

If experimental values of *in vitro* solubility in biorelevant media with known concentrations of bile salt are available, then Eq. (4) can be used to calculate the solubilization ratio. This is the preferred method for estimating solubility *in vivo*.

$$\frac{Sol_{Biorel} - Sol_{aq}}{Sc_{aq} x MWt} = SRxC_{bile} \quad (4)$$

Bile salt concentrations in individual gut compartments in fasted and fed state can be calculated based on published values for human, rat, and dog (Porter et al., 2007; Sugano, 2009a). All of these calculation methods have the advantage of avoiding large numbers of experiments to reflect the changing environment with location in the small intestine: the drawbacks are that they assume a fixed bile salt to lecithin ratio when in fact this ratio shows considerable variation within the population and that they are based on just taurocholate as the bile salt, whereas various bile salts are present in the gut and the ratios of these vary with species.

3.5.2. Accounting for the solubility/permeability interplay: the PBPK approach

As release from the dosage form and subsequent uptake into the gut wall represent processes in series, the interplay between them

can have an impact on the efficiency of absorption. As drug is taken up across the gut wall, the concentration of drug at the site of absorption will decrease, facilitating the dissolution of more drug. So if the gut wall is highly permeable to the drug and uptake occurs quickly, this will effectively create sink conditions in the lumen for dissolution and lead to efficient drug absorption, even if the drug is poorly soluble. But if the gut wall has only a low permeability for the drug, the contribution of uptake to the concentration gradient driving force for further dissolution will be minimal, dissolution will be slow and thus the efficiency of absorption will be compromised. The question is, how can this interplay of solubility and permeability be addressed best in preclinical development?

In recent years there have been a number of attempts to create *in vitro* models which couple dissolution with permeability experiments (Kataoka et al., 2012; Mellaerts et al., 2008) or use a biphasic dissolution set-up, with the additional (organic) phase intended to mimic partitioning into the intestinal membrane e.g. (Kostewicz et al., 2014; Shi et al., 2010). Some issues with these approaches include the difference in usual experimental scale, since dissolution experiments are typically performed with prototype formulations in media volumes in the hundreds of milliliters whereas permeability experiments are usually performed using small volumes of media which are compatible with the membrane (typically a monolayer cell culture or an artificial membrane). Although the biphasic set-ups overcome some of these difficulties, the question of how well an organic phase can represent intestinal permeability remains and, in addition, a new issue pops up, namely, the extent to which biorelevant media components (if used in the medium) may partition into the organic phase instead of remaining in the dissolution medium.

A potential way around the twin dilemmas of experimental scale and compatibility is to couple the dissolution with the permeability data using PBPK models. In this case, the dissolution experiments can be run under conditions closely simulating those in the GI lumen using prototype formulations, while the permeability studies can be conducted in PAMPA or cell culture monolayers under conditions that are appropriate for the respective systems. Coupling of the data in the PBPK model then proceeds via the differential equations set up to describe uptake rate as a function of the concentration gradient on hand and, *vice versa*, to take into account the effect of removal of drug across the gut wall on the dissolution rate. This approach is illustrated by a recent paper describing absorption of aprepitant from micronized and nanosized formulations using a semi-PBPK (STELLA®) model (Shono et al., 2010). In the case of micronized drug, the dissolution was very slow, even in biorelevant media, and was shown by sensitivity analysis to be the dominant influence on the plasma profile. By contrast, dissolution from the nanosized formulation was very fast and for this formulation, the sensitivity analysis revealed that the permeability is also an influential factor on the absorption of aprepitant. In a further paper (Wagner et al., 2012), the plasma profiles of a weakly basic development substance (“Compound A”) were predicted by integrating dissolution and precipitation results obtained in biorelevant media with separately obtained permeability data into a STELLA® model. In this study, permeability restrictions were introduced into the model using an absorption rate constant calculated *a priori* from the Caco-2 permeability value of Compound A, the effective intestinal surface area and appropriate intestinal fluid volumes. Although biorelevant dissolution tests proved to be helpful in predicting the food effects on Compound A absorption on a qualitative basis, the plasma profiles of Compound A could only be predicted quantitatively when the results of biorelevant dissolution test were coupled with the permeability data in the PBPK model.

It should be noted here that two different kinds of permeability restrictions can be built into PBPK models, if needed. As pointed out in Section 2.4.3, permeability can be divided up into diffusion across the ABL and uptake into the membrane and these are usually handled as sequential events (see Eq. (1)). Theoretically, either of these could be the rate limiting step to uptake, though mathematically the effect is the same. Some authors have described permeability restrictions in terms of the ABL (Shono et al., 2011; Takano et al., 2006) while others have induced a general permeability limitation effectively cutting down the uptake rate constant (Juenemann et al., 2011). Other authors have tried to cover both (Sjögren et al., 2013). Recently, Fei et al. compared the importance of the ABL and uptake for a poorly soluble lipohilic drug (fenofibrate) from a lipid-based formulation and concluded that in this case, the restriction to uptake due to the ABL was inconsequential compared to the restriction to uptake (Fei et al., 2013). Additionally, it needs to be considered the behavior of different colloidal and particulate species (such as bile, excipient micelles or nanoparticles) in the ABL which may potentially be important for predicting the GI absorption of poorly soluble drugs and enabling formulations (Sjögren et al., 2013). Going forward, it will be important to tease out the relative importance of the unstirred water layer and permeability on the uptake of drugs from the GI tract and represent this properly, both in the experimental and in the PBPK models.

3.5.3. Challenges for integration of results from in vitro release and precipitation models with PBPK modeling

3.5.3.1. Supersaturation and precipitation from “enhanced” dosage forms. Given the greater number of new drug candidates coming out of discovery that are poorly water soluble and the subsequent development of “enhanced” formulations to deal with this issue, concerns about precipitation within the GI tract as an unwanted effect following oral administration have been raised. An assessment of supersaturation and drug precipitation for poorly soluble drugs is important during formulation development. Whilst a number of different *in vitro* tests have been used in the past (e.g. (Arnold et al., 2011; Bevernage et al., 2012; Carlert et al., 2010; Gu et al., 2005; Kostewicz et al., 2004) very few studies have actually undertaken a direct *in vitro* and *in vivo* comparison (Psachoulias et al., 2012, 2011) making it difficult to assess the relevance of the *in vitro* data to the clinical setting.

Since the complexity of the supersaturation and precipitation process is influenced by the physicochemical characteristics of the drug and formulation design as well as the physiology of the GI tract, no *in vitro* test can integrate all of the important parameters affecting the GI absorption of these poorly soluble drugs. There are now more examples where results from the *in vitro* precipitation assays have been incorporated into PBPK modeling in order to provide a more holistic approach to examine the relationship between GI physiology and precipitation on the resulting absorption profile (Kuentz et al., 2006; Shono et al., 2011; Takano et al., 2010; Taupitz et al., 2013; Wagner et al., 2012). Although this is a promising approach, there are still many unknowns regarding the factors influencing precipitation within the GI lumen (such as the interplay between permeability and its influence on precipitation and re-dissolution of the precipitate) and up to date there is little consistency in terms of the *in vitro* methodologies used. A further complication in combining *in vitro* data with PBPK models lies in the different procedures used by the different commercially available PBPK models to describe precipitation (see Section 3) and the variety of ways they require *in vitro* precipitation data to be utilized within the model. In summary, further optimization of the *in vitro* methodologies to examine supersaturation and precipitation is sorely needed, and additionally a concerted effort to improve the integration of the *in vitro* data into the PBPK models in order to predict the absorption profile more accurately is warranted.

3.5.3.2. In vitro release data for dosage forms with modified release patterns. Up till now, most simulations of dosage form performance after oral administration have been performed for IR preparations and the current PBPK models accommodate *in vitro* dissolution data for such dosage forms quite well. Typically, separate dissolution rates for the formulation under gastric and upper intestinal conditions are determined in the laboratory under biorelevant conditions and the fate of the drug after administration of the formulation can be predicted by coupling these dissolution data with the physiological parameters of the GI tract (gastric emptying and volumes) and uptake parameters e.g. (Shono et al., 2009).

For delayed or ER formulations, the situation is obviously more complex, as the dosage form will be exposed to a continuously changing environment as it proceeds through the GI tract. In this case, the release rate needs to be determined as a function of the luminal composition and prevailing hydrodynamic conditions. One approach to address this need would be to determine the release rate under a variety of different conditions in individual experiments and then couple the release rates with the transit characteristics of the dosage form such as in the example illustrated by Bergstrand et al. (2012a,b). The results of such studies can be readily incorporated into most of the generic PBPK models. However, running tests in individual media does not address the possibility of carryover effects. These are known to occur e.g. with enteric coated dosage forms, for which the exposure time to acid has an influence on the subsequent release under intestinal conditions (Kambayashi et al., 2013). Another approach would be to set up a "one-step" release test, in which the dosage form is subjected to a sequence of different conditions in an attempt to mimic the passage through the GI tract in just one experiment. This can be done with standard dissolution equipment such as the European Pharmacopoeia (EP) Type 3 ("BioDis") tester or Type 4 ("Flow through") tester. While this is an appropriate strategy for developing IVIVCs between *in vitro* and mean plasma profiles using deconvolution techniques, it creates an issue for integration into PBPK models, in that the passage characteristics chosen for the *in vitro* set-up may or may not correspond to the passage characteristics employed in the PBPK model. As a result, there have only been a very limited number of papers in which modified release formulations have been described by PBPK modeling (Dokoumetzidis et al., 2007; Lukacova et al., 2009).

Fotaki et al. (2009) studied an osmotic pump dosage form and a matrix formulation using a rather rudimentary set-up with only three dissolution media (SGF_{sp}, FaSSIF or FeSSIF, and SCOF) to compare BioDis with the Flow-through set-up in terms of predicting plasma profiles in the fed and fasted states. After the data were coupled with a semi-PBPK STELLA® model, no clear advantage with either type of equipment in terms of predicting plasma profiles was evident.

More recently, Kambayashi et al., 2013 investigated the performance of enteric coated diclofenac tablets using biorelevant media and coupling the results with a STELLA®-based model. In this model, the enteric coated tablet was assumed to be emptied from stomach only in conjunction with Phase 3 activity. A virtual population was created with a range of gastric pH and emptying times. The oral PK profiles were predicted for each virtual subject individually, with lag times and dissolution adjusted according to the subject's gastric emptying time. The dissolution profiles and hence the plasma profiles were highly affected by the period of exposure to gastric conditions. Using this approach, not only the mean profiles in the fasted state but also the variability could be predicted successfully.

Going forward, to predict the plasma profiles of more challenging formulations (e.g. matrix type CR formulations and coated CR formulations) it will be necessary to identify release test protocols that not only adequately represent the continuously changing conditions in the GI tract but also are aligned properly with the passage assumptions in the PBPK model. Particularly for drugs which have site-dependent absorption and/or metabolism, the alignment of release data with PBPK model characteristics is crucial to success. The PBPK models offer the ability to create a virtual population and hence describe not only the mean behavior of the MR dosage form but also the expected range of behavior in a given patient population. In order to take full advantage of this capability, a paradigm shift in the way prototype MR formulations are studied *in vitro* during development will likely be needed.

4. PBPK models for predicting oral drug absorption: the future

4.1. How to best combine PBPK with in vitro test input: striking the right balance

From the foregoing sections it is clear that there have already been quite a few successes with PBPK models in identifying the key issues in the development of new drugs and in predicting their behavior from various dosage form options *in vivo*, as well as with applications to investigate clinical questions such as performance in populations showing enzymatic polymorphism, in special disease states and to predict drug interactions. In the early days of PBPK modeling, the strictly "bottom up" approach was touted as a way forward that would enable drug development on an entirely virtual basis. While prediction of drug behavior has succeeded to a reasonable extent, predicting the influence of the dosage form on the *in vivo* performance on a purely virtual basis has proven elusive. More recently, "middle out" approaches have been used, in this case, *in vivo* data is used to optimize or refine the existing PBPK model in a 'predict, learn and confirm' paradigm. Although there are still many aspects that need to be addressed, including improving our understanding of conditions in the lower small intestine and in the colon as well as creating more appropriate *in vitro* models for testing dosage form performance at all levels of the GI tract, the "middle out" approach appears to be the most practical way forward in today's development paradigm.

4.2. Predicting variability of response in the target patient population

Oral bioavailability, particularly for sparingly soluble and/or poorly permeable compounds and those that undergo extensive first-pass metabolism, often exhibits a high degree of between and within subject variability. Therefore, predicting the level of variability is as important as predicting mean values (McConnell et al., 2008). Since F can be affected by the age, gender, race, genetics and disease of the patient and as well as by the intake of food, only when these elements are mechanistically accounted for is it possible to obtain reliable predictions of BA.

For any drug, the degree to which the absorption processes (liberation, dissolution, absorption, metabolism and transport) are affected by physiological factors differ and depends upon the characteristics of the drug itself. For example, for a highly permeable drug, transporter heterogeneity along the GI tract (Harwood et al., 2012) may not have a significant impact while the opposite may be the case for a less (passively) permeable drug. Similarly, stomach pH variability can have a major impact upon the solubility of a sparingly soluble weak base with $pK_a \ll 7$ but gastric pH is of little or no significance for neutral compounds or highly soluble bases with $pK_a \gg 7$ (Jamei et al., 2009b). Moreover, our understanding of gastric emptying and its impact on the dissolution characteristics of solid formulations has significantly expanded over recent times through the use of imaging techniques (see for example (Koziolek et al., 2013)). These considerations of physiological variability become even more important when developing

formulations for pediatric and geriatric patient groups, for which our knowledge is currently fairly limited (Bowles et al., 2010) or for the various racial groups.

Depending upon formulation characteristics, physiological factors such as gender can also influence the variability in BA. For example, it has previously been reported that when ranitidine is dosed with increasing levels of PEG 400, an enhancement in the extent of absorption in male subjects but not in female subjects was observed. Whilst an explanation for the difference was not reported, these findings highlight the potential influence of gender-by-formulation effects on drug bioavailability. (Ashiru et al., 2008). Other impacts of gender upon BA have also been reviewed (Freire et al., 2011). Further, Koren and co-workers recently challenged the assumption of intra-subject variability being similar between males and females and argued that studies of BE of sufficient power should be undertaken in women for all generic drugs aimed at women (Koren et al., 2013).

Disease can directly affect drug absorption and disposition. For example, gastroparesis or delayed gastric emptying, linked to type 1 or 2 diabetes, can delay the absorption process. Further, as in the case of coeliac patients, the combined effect of a delayed small intestinal transit time and potential changes in intestinal permeability (Bjarnason et al., 1995; Sadik et al., 2004) may have a significant effect on BA. Permeability can also be modified in patients with other inflammatory bowel diseases, such as Crohns's disease, and intestinal infections including (amongst many) Giardia, Salmonella and malaria and by drugs themselves such as the NSAIDs (Bjarnason et al., 1995). Further, patients may take more than one drug at a time and some drugs can affect the stomach pH (e.g. proton pump inhibitors (Budha et al., 2012)) or change the stomach or small intestine motility (e.g. alcohol, anticholinergics and narcotic analgesics (Nimmo, 1976)). Therefore, improved understanding and consideration of biological and physiological feedback in response to co-administered substances is also required.

Overall, a better understanding of the intrinsic and extrinsic factors affecting GI absorption, including where appropriate, knowledge of their variability and covariates, should significantly improve our ability to predict the inter- (and potentially intra-) variability of exposure to drug. Mechanistic models provide the most appropriate framework within which this can be fully examined.

4.3. Evaluating PBPK success: which yardsticks should we use?

Before considering which metrics are most suitable for evaluating PBPK success, it is important to pinpoint the goals of applying the PBPK model. The most ambitious goal would be a full prediction of the plasma concentration–time profile after oral dosing. If data following i.v. administration is available, it should be possible to identify the disposition parameters of the drug and apply these in the model to translate gastrointestinal (solubility, dissolution, stability, motility, etc.), absorption (permeability) and first pass metabolism related parameters into a plasma profile. The same would apply when a disposition model for the drug is available in the literature. Of course, if an accurate prediction of the plasma profile is the goal, success will depend not only on the ability to model the absorption process, but also the accuracy of the disposition parameters. In particular, if disposition parameters are taken form the literature and coupled with dissolution data to predict results in a different group of subjects, it is very likely that this will lead to discrepancies between the predictions and the observed results.

Unfortunately, for many poorly soluble drugs, there is no i.v. data available. Using data derived from an oral solution to calculate disposition parameters may be inaccurate, since if precipitation in the gastrointestinal tract occurs after administration of the solution, the entire dose may not become available for absorption. In such cases, the objective of applying the PBPK model may be to simply predict the amount of drug absorbed as a function of time. These results can then be compared with absorption profiles that have been calculated by deconvolution techniques (Cutler, 1978; Fotaki et al., 2005), noting that, as indicated above for calulating disposition parameters, deconvolution techniques are most reliable when based on an i.v. data comparison. If fraction absorbed vs. time profiles can be predicted successfully, then both food effects and formulation effects can be captured by combining the appropriate drug release profiles with gastrointestinal physiology parameters in the PBPK model. Additional, key questions such as the influence of physiological and pathological variations in gastrointestinal parameters on drug performance after oral dosing can also be answered at this level of application of PBPK models. In the context of the OrBiTo project, the prediction of relative bioavailability among various formulations and the influence of dosing conditions (fed vs. fasted state) is particularly relevant, since the success of these types of predictions is crucial to optimizing formulation development.

There are several metrics which can be used to judge the quality of the predicted concentration–time profile. These metrics broadly fall into two categories: those associated with some summary parameter taken from the profile, such as AUC, and those based on the whole profile. The issue associated with using a single summary parameter such as AUC is that it does not relate to the shape of the profile. For that reason in BE assessment it is common to look at several parameters, typically AUC, C_{max} and T_{max}, which when taken together are more informative about the size and shape of the profile. Agreement can be measured in terms of how close the predicted parameter is to the observed; usually this is expressed as a relative agreement such as Fold Error (FE), defined as the ratio of the predicted to the observed (or *vice versa* depending on whether it is an over- or under-prediction), so that a FE of one represents perfect agreement.

There are several methods that have been applied to the comparison of whole profiles, particularly those used for comparing dissolution profiles (see (Vertzoni et al., 2003) and for a nonparametric test (Gomez-Mantilla et al., 2013)). Also the idea of using criteria based on FE for the whole profile has been considered for the predictive performance of interspecies scaling models (Van den Bergh et al., 2011). Regardless of the method used, a level of acceptance will need to be set and that should be based on the purpose for which the prediction is to be used.

4.4. Applications of PBPK modeling in the context of the OrBiTo project

Today, biopharmaceutical profiling at different stages of drug development is still, to a very high degree, a trial and error process, with traditional dissolution methods derived from quality control and *in vivo* testing in animals as the primary tools to guide development. BE studies in humans commonly have to be performed to verify therapeutic equivalence of the product as it moves through the different phases of clinical development, as the quality control methods are often not predictive of clinical performance. The introduction of more clinically relevant methods and the ability to link laboratory tests to patient outcomes, in the context of QbD, would go a long way to streamlining formulation and production costs as well as providing more safety and efficacy assurance for patients.

A key to achieving these objectives is to develop reliable PBPK models, which can integrate data from clinically relevant laboratory tests with human physiology, in all its variations, to predict patient outcomes. Such PBPK models should be able to anticipate the effect of pharmaceutical factors on drug absorption and the

plasma concentration profile in addition to predicting the effects of food intake or differences in system parameters due to gender, race or disease states. Whilst the effectiveness of PBPK models for simulating human plasma concentration–time profiles have been evaluated in the past (Poulin et al., 2011), one of the main objectives of the OrBiTo project will be the alignment of optimized *in vitro* test methods to capture dissolution, release from enhanced formulations and modified release formulations, the potential for precipitation in the GI tract and the interplay between release and gut wall uptake with a state of the art description of the GI physiology through the use of PBPK models. Whilst not directly a focus of the OrBiTo project, the current PBPK models could be further refined with the integration of additional functional data such as blood flows and activities of organs or tumors, which has been made available using functional MRI (van Zijl et al., 1998). Further, by considering both genomics and proteomics, this can help to refine the PBPK models with respect to the expression of specific enzymes and transporters in specific population/patient groups and thereby allow simulations to be conducted under specific conditions. Integration of these vital GI physiological and functional data into current models would allow the creation of individualized PBPK models which will facilitate prediction of absorption characteristics under different conditions. Moreover, by incorporating the variability that exists in the GI tract, the existing tools will be better able to capture between- and within-subject variability. In this context, PBPK modeling could be used prior to conducting clinical trials in humans to predict the influence of random between- and within-subject variability, in different population groups and under different dosing conditions in order to evaluate the performance of a dosage formulation. Further, rather than predicting just a single typical plasma concentration profile, PBPK modeling can be used to predict population variability which may be an important consideration for the development of new PBPK models or even in the establishment of an IVIVC (Gaynor et al., 2009; Jamei et al., 2009b; Lukacova et al., 2009; Okumu et al., 2008; Polak, 2008).

The use of PBPK-PD models could drastically change the way the pharmaceutical industry develops drugs and the way the regulators accept them. It would shift the paradigm from a statistical approach to establishing efficacy, toxicity or product equivalence, to a more mechanistic and deterministic one. Formulation development would become more patient outcome-focused as the formulation would be developed with the therapeutic target as the main driver. The ability to predict changes in pharmacokinetics and thus pharmacodynamic response in different patient subgroups could substantially reduce the amount of clinical testing needed to support a drug approval process. Importantly, coupled with physiologically relevant *in vitro* tests, regulatory relief in the form of PBPK-based biowaivers may become the norm in the future, obviating the need for expensive and time-consuming bridging studies late in clinical development. At the very least, with the benefit of PBPK models, it will be possible to develop robust design spaces for oral pharmaceutical dosage forms and thus truly achieve the goals of "quality by design".

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