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Leveraging the use of *in vitro* and computational methods to support the development of enabling oral drug products: An InPharma commentary

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ABSTRACT

Due to the strong tendency towards poorly soluble drugs in modern development pipelines, enabling drug formulations such as amorphous solid dispersions, cyclodextrins, co-crystals and lipid-based formulations are frequently applied to solubilize or generate supersaturation in gastrointestinal fluids, thus enhancing oral drug absorption. Although many innovative *in vitro* and *in silico* tools have been introduced in recent years to aid development of enabling formulations, significant knowledge gaps still exist with respect to how best to implement them. As a result, the development strategy for enabling formulations varies considerably within the industry and many elements of empiricism remain. The InPharma network aims to advance a mechanistic, animal-free approach to the assessment of drug developability. This commentary focuses current status and next steps that will be taken in InPharma to identify and fully utilize 'best practice' *in vitro* and *in silico* tools for use in physiologically based biopharmaceutic models.

1. Introduction

The pharmaceutical development process of developing molecules into medicines is wrestling with considerable changes. The drivers of change include the need for continuous innovation in the emerging healthcare landscape, such as enabling technologies for increasingly complex drug discovery pipelines. In addition, some of the drivers of change reflect the evolving societal needs such as more affordable medicines or a need for more ethical pharmaceutical development including environmental sustainability or reducing animal testing. These complex and multifactorial challenges necessitate public–private partnerships that foster collaboration. The InPharma network is an EU Horizon 2020 funded European Industrial Doctorate programme that brings together multiple industrial and academic partners, with a collective goal of developing a fully integrated, animal-free, end-to-end model-based approach to oral drug development.

Research in InPharma is focused on linking data from drug profiling repositories with computational pharmaceutics approaches to inform optimal enabling formulation strategy and predict drug-excipient selection for a specific drug candidate. Once the prototype formulations

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have been manufactured, the next step in the end-to-end modelling approach will involve generating biorelevant *in vitro* tests customized to predict performance of enabling formulations under clinically relevant scenarios. Finally, by integrating biorelevant *in vitro* data into physiologically based biopharmaceutics (PBB) *in silico* models, the goal will be to replace pre-clinical oral pharmacokinetics studies in animals with virtual trials run in PBB models to predict the bioavailability of prototype drug products in humans. Therefore, the InPharma network's aim is to unlock the potential of computational modelling and customized laboratory-based technologies, leading to more scientifically and ethically justifiable approaches to predict drug absorption from 'enabling' oral drug formulations. 'Enabling formulations' are broadly defined as oral drug products which aim to supply the luminal contents with the drug either in solubilized or supersaturated state. The focus on oral enabling formulations reflects the immense challenges faced by the pharmaceutical industry in developing formulations of poorly water-soluble drug candidates that ensure maximal systemic exposure.

The research in InPharma builds on the success of previous EU funded consortia. Specifically, Dressman and colleagues developed a refined development classification system ('rDCS') as part of the industry-academic IMI OrBiTo partnership, to guide decisions about the developability of oral drug candidates (Rosenberger et al., 2018). The InPharma project will build on this work by fully integrating the rDCS classification approach across a more extensive range of drugs and comparing the success rate of selecting the formulation type via application of rDCS relative to previously reported absorption studies in animals. In the EU H2020 funded PEARRL project, initial efforts to design formulations based on *in silico* calculations of drug-excipient interactions proved promising. The InPharma project aims to expand this research by developing new computational pharmaceutics tools to take the guesswork out of choosing the right excipients for the selected enabling formulation strategy. Finally, within the OrBiTo Project *in vitro* tools were developed, in large part to predict performance of conventional dosage forms in fasted healthy adult volunteers. InPharma aims to customize these *in vitro* tools and integrate the data into PBB modelling to predict the performance of enabling formulations under various conditions of administration.

1.1. Implementation of rDCS to better understand the formulation challenge

The Biopharmaceutics Classification System (BCS), first proposed in 1995, has often been used to guide drug development (Food and Drug Administration (FDA), 1995). However, the BCS was intended to be applied as a regulatory tool to classify drugs in terms of solubility and permeability to determine whether the need for clinical bioavailability/bioequivalence studies in generic drug development of immediate release oral solid dosage forms could be waived. For this reason, the BCS classification scheme is rather conservative and provides little guidance on appropriate formulation strategies for candidates in development. As a result, formulation development guided by BCS classification often becomes a trial-and-error exercise that results in testing of numerous prototypes in animals until a usable formulation is found.

To address the need for a tool to help guide oral small molecule formulation development, the DCS was introduced in 2010 (Butler and Dressman, 2010) and a refined version rDCS was proposed in 2018 (Rosenberger et al., 2018) to build upon the concepts in the DCS. The main differences between the BCS and rDCS for standard investigations are summarized in Table 1. Importantly, the divisions between high and low solubility and permeability are defined in a way that is more conducive to decision-making in a development setting. Additionally, customized investigations are introduced for certain classes of drug candidates.

The standard investigations are conducted to provide an initial—and for some drug candidates—a final developability assessment of the candidate based on the dose range and its solubility and permeability characteristics. Customized investigations may be triggered when specific criteria from the standard investigations are met. These customized investigations include small scale dissolution experiments and small-scale supersaturation or precipitation experiments, as described in Table 2 (Rosenberger et al., 2018).

Several case histories illustrate the utility of rDCS to classify a drug candidate—especially over a range of doses—and to visualize the effect of different types of formulations on the likelihood that a successful marketable product can be produced (Rosenberger et al., 2019). For example, an rDCS analysis was applied to the marketed drug acyclovir (an ampholytic antiviral compound) using literature data for solubility and permeability. During the standard investigation, the upper limit of the dose range was adjusted to 800 mg, corresponding to the highest single dose recommended in the Prescriber Information (GlaxoSmithKline (Ireland) Limited, 2021). The rDCS classification for acyclovir was identified as class III (good solubility but poor permeability) for the 5 mg and 50 mg doses, and class IV (poor solubility and permeability) for the 800 mg dose. Had this been an investigational compound, the development team would have seen, based on the rDCS classification, that a strategy to improve the permeability of the compound would be needed to increase its bioavailability. One approach to help improve permeability of compounds is to create a prodrug of the original molecule. In the case of acyclovir, conjugation of the amino acid valine to create valacyclovir led to an improvement in permeability as valaciclovir is a substrate for amino acid transporters (MacDougall and Guglielmo, 2004).

1.2. Leveraging in vitro methods with PBB modelling for the development of enabling drug formulations

Although *in vitro* models for prediction of the *in vivo* performance of oral dosage forms have been proposed in recently completed EU-funded projects, *e.g.*, OrBiTo (Butler et al., 2019) and PEARRL (O' Dwyer et al., 2021) the focus in those projects was on conventional or modified release products, rather than on enabling drug products. Scaling from *in*

Table 1
Summary of the differences between BCS, DCS and rDCS (Rosenberger et al., 2018).

Input	BCS	DCS	rDCS
Solubility	Buffer pH 1-6.8	Fasted State Simulated Intestinal Fluid (FaSSIF) solubility	Estimated FaHIF solubility based on correlation between solubility in FaHIF and selected test medium (*e.g.*, FaSSIF)
Dose	Dose or Expected dose to calculate D/S	Dose or Expected dose to calculate D/S	Determine D/S for 5 mg, 50 mg, and 500 mg dose level when dose is not yet known
Effective Volume to dissolve the dose	250	500	500
Classes	Four (I-IV)	Five (divides class II into IIa and IIb)	Five (divides class II into IIa and IIb)
Permeability	85% Fa	Effective human permeability (can be estimated using in-vitro studies)	Estimated effective human permeability based on correlation between in-house method and published results in humans
Custom Investigations	None	None	Triggered when certain criterion from Standard investigations are met (*e.g.*, for weak bases and salts of weak acids)

Table 2
Overview of the customized investigations specified by the rDCS (Rosenberger et al., 2018).

Trigger	Investigation	Rationale
rDCS class I, IIa and/or III, but aqueous solubility < 100 ug/mL	Small scale dissolution experiment	Confirm true intestinal dissolution characteristics (fast dissolution needed for sufficient bioavailability)
Weak base with a rDCS class IIb or IV	Small scale supersaturation/ precipitation experiment	Weak bases will have their highest solubility in the low pH environment of the stomach leading to supersaturation, but carry a risk of precipitation in the higher pH environment of the small intestine
Salt form for a weak acid from rDCS class IIb	Small scale supersaturation/ precipitation experiment	Weak acids formulated as salts may precipitate as the free acid at the low pH of the stomach.

vitro data and from preclinical data to the *in vivo* behavior in humans was shown to be error-prone (Ahmad et al., 2020), underlining the need for advances in PBB modelling. Much progress in PBB modelling was achieved within those projects, paving the way to address the same issues for enabling drug products in InPharma.

1.3. Computational methods

The availability and 'horsepower' of *in silico* computational methods has greatly increased in recent years. Although this is a very broad field, in the InPharma project the emphasis is on three subsets of computational modelling: the first is PBB modelling based on mechanistic differential equations, the second consists of computational methods to support biopharmaceutical *in silico* predictions, and the third comprises *in silico* approaches to understanding drug-excipient interactions.

PBB modelling can be loosely defined as using biopredictive *in vitro* data with physiologically based pharmacokinetic (PBPK) modelling to predict drug absorption and pharmacokinetics, usually after oral dosing (Pepin et al., 2021). Methods supporting PBB modelling include estimating physicochemical characteristics such as pKa, logP, or solubility values. Such estimates are particularly crucial in an early development stage when only limited experimental data are available. While this commentary provides an overview of *in silico* tools for prediction of physicochemical properties, the reader is also referred to other review articles for more detailed information on *in silico* prediction of any given drug characteristic, for example, in case of predicting drug solubility (Kuentz and Bergström, 2021).

Computational pharmaceutics can also help with selection of a formulation approach or to guide formulators in their choice of excipients. Therefore, this article also discusses different calculation methods for this purpose once the bio-enabling formulation principles are defined. Especially in the case of cyclodextrin (CyD) formulations, much effort has been invested in various algorithms to study the intended molecular complexes. However, it is still a scientific challenge to computationally predict interactions in a reliable manner for most drug-excipient combinations and it is regarded as an emerging field. Meeting this challenge would not only support the design of optimal enabling formulations, but could also be used to inform PBB modelling. To learn more about efforts to date and the gaps that remain, a commentary published by the EU-funded project PEARRL discussing algorithms in selecting bio-enabling formulations is recommended (Kuentz et al., 2021).

1.4. Structure and aims of this commentary

This commentary consists of two main parts:

In the first part, *in vitro* methods that have been applied in the development of enabling drug products by considering PBB modelling approaches are summarized and discussed. Six classes of enabling drug products are considered: a) amorphous solid dispersions (ASDs), b) lipid-based formulations (LBFs), c) drug complexes with CyDs, d) co-crystals and salts, e) deep eutectic solvents (DES) and (f) and nanocrystal systems. Where appropriate, examples of the incorporation of *in vitro* data into PPB models are shown.

In the second part, currently employed computational methodologies for informing PBB modelling of enabling drug products are presented, specifically in regard to estimating biopharmaceutics parameters that are relevant to enabling drug products and recent methods proposed to account for drug-excipient interactions computationally.

In both parts, emphasis is given to highlighting the gaps in understanding. At the end of the commentary, the roadmap to be followed in InPharma for closing at least some of these gaps is laid out.

2. To what extent *in vitro* methods have been useful to date in the development of enabling drug products by considering PBB modelling approaches?

2.1. Amorphous solid dispersions (ASDs)

ASDs are a class of enabling formulations that are usually prepared with the intent of increasing the solubility and/or dissolution rate of an active pharmaceutical ingredient (API). Ideally, an ASD is a molecular dispersion of the API in an amorphous carrier, in other words a glassy solution (Van den Mooter 2012). Crystallization of API is hindered by the carrier, which is typically a hydrophilic polymer (Bellantone 2014). The solubility/dissolution benefit achieved by ASD formulation is a result of multiple factors, such as improved wettability of the drug in the presence of the polymer, the excess free energy of the amorphous form compared to the corresponding crystalline state, separation of individual drug molecules by polymer chains, and subsequent prevention of drug precipitation upon contact with aqueous media (Laitinen, 2014). To minimize pill burden, the highest possible drug loading is targeted in the formulation. In many cases API loadings up to 30–40% can be obtained without phase separation immediately after preparation, however, this highly depends on the selected API, carrier, and processing method (Van den Mooter 2012).

ASDs generally dissolve rapidly, driving dissolved API concentrations to the amorphous kinetic solubility limit. During dissolution, phase separation can be observed in the form of sub-micron amorphous particles (Tho et al., 2010), which subsequently can be the root cause of true supersaturation (Frank et al., 2012) and enhanced permeation (Frank et al., 2014). This phenomenon has been called liquid–liquid phase separation (LLPS) (Taylor and Zhang, 2016). Potentially, after a lag time, crystalline precipitation may occur. Thus, appropriate *in vitro* experiments can inform the modeller about the interplay between phase-separation and supersaturation, and thus the stability of the supersaturation. If the solutions remain stable *in vitro* over the intestinal residence times then it may be assumed this applies *in vivo* and the PBB model can be parameterized accordingly (Arora et al., 2020; Emami Riedmaier et al., 2018). Thus, the selection of an appropriate *in vitro* methodology is crucial to *in vitro* evaluation of an ASD formulation (Newman et al., 2012). Currently, both small-scale and large-scale setups are available to assess performance of oral drug formulations.

2.1.1. Small-scale setups

To date, two small-scale, two-stage setups are commercially available: a biphasic system and a Dissolution-Permeation (D-P) system. The biphasic system (InForm platform, Pion Inc.) has been used for the evaluation of ritonavir (Norvir®) and itraconazole (Sporanox®) ASD products (Jankovic et al., 2019; O'Dwyer et al., 2020b, 2022). O'Dwyer et al. (2020b) and has been shown to be useful for estimating the precipitation rate constant (PRC) of two ASD of weakly basic drugs, itraconazole and ritonavir, estimated from the partition rate into the

decanol layer. By inputting the estimated first-order PRC into PBB modelling software (Simcyp™), the biphasic system helped to predict the performance of Sporanox® (O'Dwyer et al., 2020b). In the Norvir® study (O'Dwyer et al., 2022), input of the PRC from the biphasic system into the Simcyp™ PBB model predicted similar results for a hypochlorhydric and normal gastric environment. The simulations correlated well with published *in vivo* data for the ASD formulation, Norvir® (van den Abeele et al., 2020).

In a recent study (Tsakiridou et al., 2022), three different modified release formulations of tacrolimus were investigated using the biphasic system. Based on the total exposure of the three formulations, the system failed to predict product related differences between Envarus® and the test formulation, compared to Advagraf®. The authors explained this discrepancy by noting that particles from the Advagraf® formulation floated on the surface of the aqueous media, leading to direct mixing with the decanol layer and transfer of the entire dose into that layer within a few minutes (Tsakiridou et al., 2022).

The D-P system (μFLUX, Pion Inc.) has also been used for the evaluation of ritonavir (Norvir®) and itraconazole (Sporanox®) and tacrolimus (Advagraf® and Envarus®) ASD products (O'Dwyer et al., 2020b, 2022; Tsinman et al., 2018; Tsakiridou et al., 2022). This method consists of two chambers separated by an artificial biomimetic membrane (polyvinylidene fluoride, coated with 25 μL of a lipid solution) to simulate the transfer from donor (intestinal) to acceptor (absorbed) compartment (O'Dwyer et al., 2019). In the donor chamber, the change from gastric to intestinal conditions is represented by the addition of concentrated FaSSIF V2 (to produce a final pH of 6.8) to dilute HCl (pH 2). Based on the concentrations in the acceptor chamber, the results matched the rank-order of the *in vivo* AUCs of the itraconazole formulations tested and correctly predicted the effect of hypochlorhydria on the ritonavir ASD formulation performance. For the tacrolimus formulations, based on the percentage of dose in the acceptor chamber at 1 h, the difference between Advagraf® and Envarus® was predicted correctly. However, the D-P system also failed to predict product related differences in total exposure (Tsakiridou et al., 2022). One limitation of such small-scale two-stage D-P systems is the small area-to-volume ratio ($1.54\ cm^2/20\ cm^3 = 0.077\ cm^{-1}$). This is problematic in the context of D-P testing, as a small permeation area can lead to permeation being the rate-limiting step, making it difficult to predict the influence of the dissolution kinetics of the formulation on absorption *in vivo*. If such sink conditions are not achieved in the experimental setup, the concentration in the donor compartment will build up, slowing dissolution of the API and potentially promoting precipitation. This will lead to underestimation of the ASD performance *in vivo* (Hate et al., 2017). Also, partition across the membrane can occur during the gastric phase of the experiment for compounds which are not ionized in the gastric environment (O'Dwyer et al., 2020b).

Both methodologies include a rapid shift from the gastric to the intestinal environment, which differs from the *in vivo* environment and may lead to substantial overestimation of precipitation (O'Dwyer et al., 2021). Incorporation of a more gradual shift from gastric to intestinal conditions is a challenge to be addressed in the next versions of the setups. Real-time analytics provides a general advantage, but *in situ* fibre optic UV probes are limited by low drug absorbence in the UV/VIS range and absorption by excipients at the same wavelength as the drug (O'Dwyer et al., 2020b). Techniques to enable robust quantification of the aqueous phase/donor compartment drug concentrations would provide additional valuable information, enabling more accurate calculation of precipitation rates, which can be considered a limitation of the current setup.

The PermeaLoop™ is an alternative D-P *in vitro* tool developed to account for simultaneous dissolution and permeation processes (Sironi et al., 2018). The high area-to-volume ratio of 1.38 cm^{-1} enables scenarios in which dissolution from the dosage form rather than permeation rate is rate-limiting to be studied. A biomimetic membrane (*e.g.*, Permeapad®) or a cellulose membrane is placed between the cells and the donor medium is pumped into a permeation cell, with the acceptor medium flowing concurrently on the other side of these membranes. This setup also operates on a small scale, with volumes of 20 mL in the donor and 35 mL in the acceptor reservoir. Both media are continuously circulating back to their respective reservoirs. Sampling is performed from the reservoirs (Sironi et al., 2018). The PermeaLoop™ setup was used to evaluate a hot melt extruded ASD containing the research compound ABT-869 (Sironi et al., 2018). A clear *in vitro – in vivo* relationship has been established using biorelevant media (FaSSIF) for Posaconazole drug products including an ASD (Holzem et al., 2022). In some cases, material adhesion on metal surfaces as well as in the tubing of the setup might lead to clogging and inconsistent results (Eriksen et al., 2020). Although the PermeaLoop™ setup achieves an advantageous area-to-volume ratio of 1.38 cm^{-1}, it still does not match exactly the physiological level which was estimated to be between 1.9 cm^{-1} and 2.3 cm^{-1} (Mudie et al., 2012).

The Permeapad® plate is a 96-well high-throughput two compartment microplate, containing a top-well (acceptor) and bottom-well (donor) separated by a Permeapad® biomimetic barrier or a cellulose hydrate membrane (PermeaPlain® Plate). The latter has been used to evaluate a freeze-dried tadalafil ASD (Jacobsen et al., 2019). This D-P setup is carried out by dispersing the ASD formulation in the bottom well medium, and then incubating the plate along with the dialysis membrane and top well, which contains the acceptor medium. After incubation, the top and bottom wells can be sampled and analyzed off-line (Jacobsen et al., 2019). Due to its miniaturized D-P setup, Permeapad® microplate setup is very efficient in terms of material usage using a total of only 300 μL medium per sample and facilitates a high number of simultaneous samples. Using FaSSIF as the medium, an initial correlation to *in vivo* literature data revealed promising results when comparing permeated concentration of drug vs. area under the curve (AUC) in rats (Jacobsen et al., 2019). However, in this system the area-to-volume ratio is noticeably lower than either the PermeaLoop™ or estimations based on the physiology. With an area available for permeation of 0.15 cm^2, an area-to-volume ratio of 0.5 cm^{-1} is achieved (Jacobsen et al., 2020). In addition, an issue with the use of plastic material on the Permeapad® plate is that some API might be lost due to non-specific adsorption onto the surface.

The PermeaLoop™ and the Permeapad® plate are promising D-P methods in terms of high throughput setups and *in vivo* correlations. To date they have not been evaluated in combination with PBB modelling approaches for any ASD formulations.

2.1.2. Large-scale setups

The USP apparatus II (rotating paddle dissolution apparatus) is a simple, well-known and standardized apparatus that can be used with varying media volumes, as well as selection of the temperature and the stirring rate of the paddle (Mann et al., 2017). Kambayashi et al. (2019) used the USP II paddle apparatus to test ASDs of a research compound 'T2CP'. Single-stage dissolution experiments were conducted in 300 mL of biorelevant media (Fasted State Simulated Gastric Fluid (FaSSGF) and Fasted State Simulated Intestinal Fluid adapted for dogs (FaSSIFc)). Dissolution and precipitation parameters were calculated based on the dissolution profiles, which were then incorporated into an *in silico* model. The modelled plasma profile concentrations for the T2CP ASDs closely matched the *in vivo* data in dogs.

The USP apparatus II has also been applied to test ASDs by mimicking the transfer from gastric to intestinal conditions. In this setup, two USP apparatus II vessels are connected via a peristaltic pump, and biorelevant dissolution experiments are conducted to simulate transfer of the API from a gastric into an intestinal compartment – the so-called Transfer Model (Kostewicz et al., 2004). This transfer model was used to evaluate the performance of a ritonavir ASD (Norvir®, Fiolka et al., 2020). In addition, the ritonavir ASD was also evaluated in this study using a two-stage dissolution experiment set-up, in which the dosage form was allowed to disintegrate and dissolve for 30 min in the

gastric medium before 250 mL double concentrated intestinal fluid was 'dumped' into the gastric medium. The results from these experiments were able to successfully capture the dissolution and precipitation kinetics as input parameters for a PBB model using Simcyp™.

A publication by Mitra et al. (2016) presented the successful prediction of the bioperformance of a spray-dried ASD of developmental molecules using GastroPlus®. A dissolution test, including a gastric step in simulated gastric fluid (SGF) and an intestinal step using FaSSIF, was performed to obtain pH-dependant amorphous solubilities and to assess the tendency for precipitation. Moderate supersaturation and a negligible tendency for precipitation was found for two of the compounds. The subsequent implementation of the amorphous solubility in GastroPlus® led to successful predictions of plasma exposure in beagle dogs over a wide dose range.

The biorelevant gastrointestinal transfer (BioGIT) system is an extension of the transfer model, simulating the transfer of gastric contents through the upper small intestine after administration of the drug with a glass of water consisting of three compartments (Kourentas et al., 2016b). The BioGIT was successfully used to estimate luminal concentrations of itraconazole ASD (Sporanox®) and ritonavir (Norvir®). Additionally, it was able to detect differences in early exposure after administration of fenofibrate ASD products in adults (Kourentas et al., 2016c; van den Abeele et al., 2020, 2018; Kostantini et al., 2023a). Tsakiridou et al. (2022) used the BioGIT to test two different types of modified release tacrolimus formulations. Envarsus® was not tested because it is non-disintegrating. Thus, only Advagraf® and the test tablet were tested. As in the small-scale setup described above, Adagraf® tended to float on the gastric media surface and adhere to the glass walls once the transfer to the duodenum began. This was attributed to the hydrophobic nature of tacrolimus and resulted in concentrations below the limit of quantification in the duodenal compartment (Kourentas et al., 2016a). Limitations of the BioGIT system may include the potential overestimation of the extent of supersaturation in the upper small intestine of very highly lipophilic APIs in ASDs (Kourentas et al., 2016c).

The TIM-1 model consists of four interconnected compartments simulating different segments of the upper gastrointestinal (GI) tract: stomach, duodenum, jejunum and ileum. Hollow fibre filters are connected to the jejunum and ileum compartments Samples of these filtrates are analyzed for API to estimate the amount of dissolved drug available for intestinal absorption *in vivo* (Barker et al., 2014). This amount is compared with the dose applied to calculate the "bio-accessible" fraction (Brouwers et al., 2011).

The TIM-1 system was used to evaluate the performance of a ritonavir ASD (Norvir®) under normal and hypochlorhydric conditions. In this case, samples taken directly from the duodenal compartment were analyzed for ritonavir concentration and these were shown to be comparable to *in vivo* concentrations (Van den Abeele et al., 2020). It is also possible to sample directly from the gastric compartment to allow the user to estimate the dissolved drug concentration at various points in the GI tract. The advantage of using a system such as TIM-1 is that a large amount of information regarding the formulation performance can be predicted for the different areas of the GI tract. However, the use of the TIM-1 system requires a lot of preparation and time, as many different media and titrants have to be prepared and the system has to be programmed, so this method is low-throughput (Van den Abeele et al., 2020). To date, no ASD dissolution data from the TIM-1 has been evaluated by PBB modelling.

López Marmol et al. investigated Sporanox® capsules under different meal intake and gastric pH modifications using a modification of the TIM: the tiny-TIM (López Mármol et al., 2022). It contains a single small intestinal compartment followed by a filter and is designed to increase throughput. API concentrations in the filtered samples were used to define the bioaccessible drug fraction for the corresponding time interval. Tiny-TIM was able to reproduce the difference between the ASD capsules and solution in terms of total exposure, and the effects of gastric pH modification on itraconazole bioavailability. However, it overestimated the positive food effect. Although simpler in design than the TIM-1, the Tiny-TIM is still a complex, time-intensive and expensive alternative in early drug development and, as with TIM-1, limitations may be that the absorption is mimicked by simple filters and that API may adsorb on the filter.

2.2. Lipid-based formulations

In LBFs, also known as Lipid-Based Drug Delivery Systems (LBDDS), the drug substances are dissolved in a vehicle consisting of lipids, surfactants and/or cosolvents. Formulation as an LBF can therefore bypass the drug dissolution stage leading to potential enhancement of oral bioavailability (Mu et al., 2013; Chakraborty et al., 2009). The most notable improvements in bioavailability for LBFs have been observed for highly lipophilic drugs (*i.e.*, "grease-ball" molecules), but more recent studies have also shown that LBF may offer advantages for highly hydrophobic drugs with high energy crystal lattices (*i.e.*, "brick-dust" molecules) (Holm, 2019; Koehl et al., 2019). Oral intake of LBFs triggers the release of bile into the intestine, which contributes to the solubilization of poorly soluble APIs by forming mixed micelles (Ilie et al., 2020; Müllertz et al., 2010). Other factors contributing to the beneficial effects of LBFs include lipid digestion as a release mechanism and the size of the dispersed particles after contact with luminal contents, with more rapid absorption associated with smaller droplets (Pouton, 2000, 2006). LBFs are a broad group of formulations that span from solutions in pure oil to systems consisting of surfactants and cosolvents only. To enable a comparison of LBFs, Pouton put forward a Lipid Formulation Classification System (LFCS) (Pouton, 2006), which divides LBFs into five different types depending on the lipid, surfactant, and cosolvent fractions in the formulation as well as the type of surfactant (Table 3).

In vitro tests for LBFs are typically divided into *in vitro* dispersion tests and *in vitro* digestion (lipolysis) tests.

2.2.1. In vitro dispersion tests

In vitro dispersion tests typically are run in official dissolution test apparatus such as the USP apparatus II (paddle) (Feeney et al., 2016). Usually, these investigations are performed in biorelevant media, for example FaSSGF or FaSSIF (Feeney et al., 2016; Griffin et al., 2014). The ability of the LBF to disperse in the medium as well as the propensity for drug precipitation upon dilution are studied (Feeney et al., 2016; Fei et al., 2013). For LBFs that are non-dispersing and/or require a digestion step to enhance luminal drug solubility, such as LFCS Type I systems, the *in vitro* dispersion tests are not considered suitable by some authors (Porter and Charman, 2001). However, Fei et al. reported a good rank order correlation between *in vitro* dispersion testing and *in vivo* performance in healthy human volunteers across several different fenofibrate Type IIIA LBFs and a capsule containing micronized API powder using the dispersion approach (Fei et al., 2013). Similarly, for Type III LBFs it was shown that a simple dispersion test correctly predicted differences

Table 3
Lipid formulation classification system showing typical composition of various types of lipid formulations (Pouton, 2006).

Excipients in formulation	Content of formulation (%, w/w)				
	Type I	Type II	Type IIIA	Type IIIB	Type IV
Oils: triglycerides or mixed mono and diglycerides	100	40–80	40–80	< 20	–
Water-insoluble surfactants (HLB < 12)	–	20–60	–	–	0–20
Water-soluble surfactants (HLB > 12)	–	–	20–40	20–50	30–80
Hydrophilic cosolvents (*e.g.*, PEG, propylene glycol, transcutol)	–	–	0–40	20–50	0–50

in the *in vivo* performance of three fenofibrate-loaded LBFs (a long chain IIIA LBF, a medium chain IIIA LBF, and a IIIB/IV LBF containing surfactants only) in landrace pigs, unlike an *in vitro* digestion test (Griffin et al., 2014).

2.2.2. *In vitro digestion tests*

The intraluminal behavior of LBFs is affected by several processes. The lipid components undergo digestion, facilitating release of the API, after which the API may be solubilized in micelles resulting from bile secretion and/or lipid digestion. If solubilization is insufficient, the API may precipitate (Porter and Charman, 2001). These processes can be simulated *in vitro* by lipolysis models. Typically, lipase (usually as part of a porcine pancreatin extract), bile salts and calcium are added to a reaction vessel containing the LBF in biomimetic media (Zangenberg et al., 2001) at 37 °C. As a result of the enzymatic reaction, free fatty acids are produced and the pH decreases. Experimentally the pH is held constant by titration with sodium hydroxide using an autoburette (Fig. 1) (Feeney et al., 2016).

Based on the amount of sodium hydroxide consumed, the extent of digestion can be estimated. Additionally, by taking samples at various time points, API distribution among the aqueous micellar phase, oil phase (undigested lipids) and the pellet (which consists of precipitated API and calcium salts of free fatty acids) can be monitored (Feeney et al., 2016). Since results can vary greatly with the specific experimental conditions, an attempt to standardize the *in vitro* testing methods for LBFs was made within the Lipid Formulation Classification System Consortium (Williams et al., 2012a, 2012b, 2013; Williams et al., 2014; Bakala-N'Goma et al., 2015; Sassene et al., 2014). Simple modifications to increase the throughput of the lipolysis model have been suggested *e.g.*, by increasing the buffer capacity to maintain the pH during lipolysis (Mosgaard et al., 2015) without the need for titration. This modification has also been implemented in a 96-well plate model (Mosgaard et al., 2017). The API distribution from the investigated LBF in the buffered lipolysis model was reported to be in line with the API distribution in a classical lipolysis model (Mosgaard et al., 2015).

The *in vitro* digestion (lipolysis) model should be regarded as a physiologically based attempt to mimic the impact of digestion process on the luminal behavior of orally ingested LBFs. Historically, however, the results have not always reflected the *in vivo* data. Feeney et al. (2016) reported a mismatch between drug precipitation observed *in vitro* and the respective *in vivo* behavior, with only half of the APIs studied (four of eight) showing a correlation between the *in vitro* and the *in vivo* data (Feeney et al., 2016; Dahan and Hoffman, 2007; Cuine et al., 2007; McEvoy et al., 2014; Larsen et al., 2013; Griffin et al., 2014; Heshmati et al., 2014). One possible reason may be the formation of an amorphous precipitate that is easily re-dissolved, as was shown for cinnarizine (Sassene et al., 2010). Another potential reason is that in the original pH-stat lipolysis model for APIs the contribution of gastric lipase to the overall lipolysis had been neglected, whereas gastric digestion accounts for almost 20% of total lipid digestion (Carriere et al., 1993). To correct this discrepancy, LBFs were investigated in an *in vitro* lipolysis model simulating the fasted and fed states by Christophersen et al. (2014). The investigation consisted of a gastric lipolysis stage, after which a duodenal lipolysis was simulated in the same vessel. The fasted state model successfully reproduced the *in vivo* performance of cinnarizine formulations. Other *in vitro* models, in which a gastric compartment is connected to an intestinal compartment via a pump, have also been proposed (Siqueira Jørgensen et al., 2018; Huang et al., 2021).

In addition the lack of an absorptive step is likely to result in an overestimation of *in vivo* precipitation. A lipolysis-permeation setup for a simultaneous investigation of lipid digestion and permeation was proposed by Bibi and coworkers using the 'Permeapad® setup (Bibi et al., 2017). Klitgaard et al. (2021) used the Permeapad® in a lipolysis-permeation experiment, whereby *in vitro* lipolysis of cinnarizine formulations was followed by *in vitro* drug permeation in Franz diffusion cells equipped with Permeapad® barriers. A linear relationship ($R^2 = 0.92$) was obtained when comparing the AUCs of the *in vitro* permeated drug and the rat plasma concentration–time profile, whereas

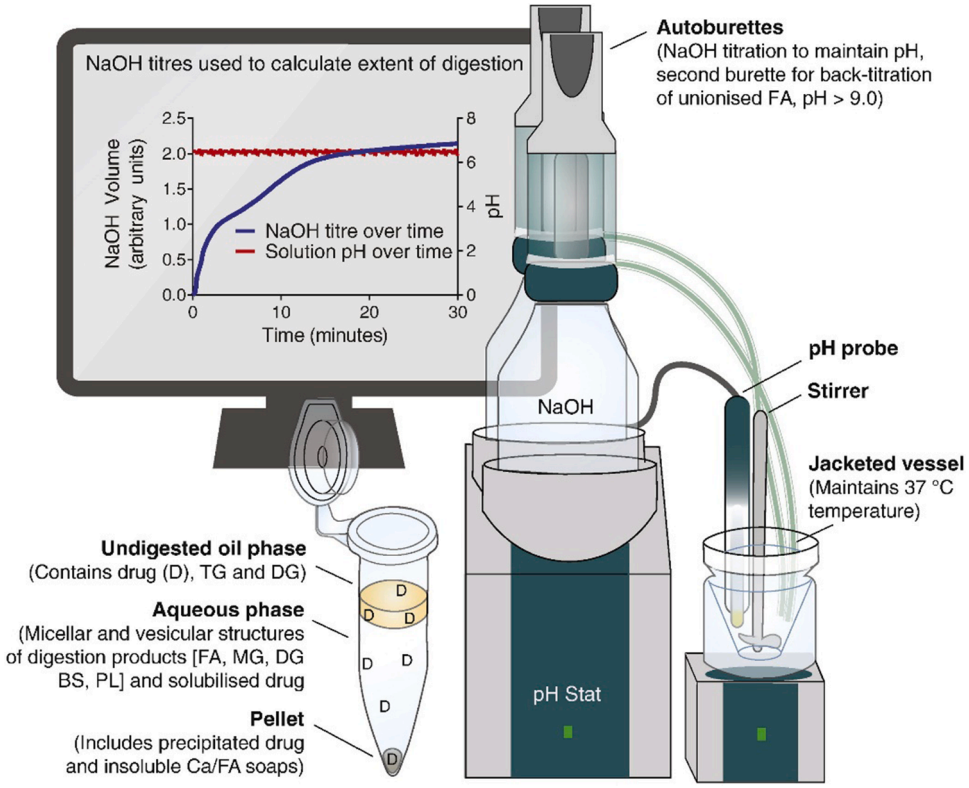

Fig. 1. pH-stat lipolysis model for the *in vitro* assessment of lipid-based formulation. Abbreviations: Drug (D), fatty acid (FA), monoglyceride (MG), diglyceride (DG), triglyceride (TG), bile salts (BS), phospholipids (PL), calcium (Ca) (Reproduced with permission from Feeney et al., 2016).

no correlation was found between the *in vivo* results and drug solubilization profiles obtained during *in vitro* intestinal lipolysis. Caco-2 cell monolayers have also been used in a lipolysis-permeation setup (Keemink et al., 2019) by including an immobilized lipase to make the setup compatible with Caco-2 cell monolayers. In contrast to the common pH-stat lipolysis model, the device successfully reproduced the *in vivo* exposure of fenofibrate-loaded LBFs, as indicated by drug transfer across the Caco-2 membrane.

Other membranes have also been used in combined lipolysis and permeation approaches. For example, PAMPA derivatives in the form of self-prepared, lecithin-based membranes on a PVDF filter support were shown to be compatible with the conditions in the digestion environment (Hedge and Bergström, 2020). Recently, it has been shown that lecithin-based membranes on a PVDF filter support resulted in data similar to Caco-2-cells for fenofibrate-loaded LBFs in a lipolysis-permeation model (Keemink et al., 2022). Falavigna et al. recently investigated a combination of the mucus-PVPA (Phospholipid Vesicle-based Permeation Assay) *in vitro* permeability model with lipolysis to predict the bioavailability of fenofibrate-loaded LBFs. The model was able to reproduce the *in vivo* performance of the formulations in rats, in contrast to the pH-stat lipolysis model (Falavigna et al., 2021a). Mucus-PVPA barriers have also been combined with the high-throughput model mentioned earlier (Mosgaard et al., 2015; Falavigna et al., 2021b). One challenge associated with these lipolysis-permeation setups is ensuring an adequate surface area to avoid permeation becoming the rate-limiting step to allow for a reliable prediction of how the dissolution kinetics of the formulation influence absorption in the GI tract.

Another experimental methodology that attempts to come closer to the *in vivo* situation in humans is by combining *in vitro* lipid digestion with *in situ* intestinal perfusion in anaesthetized rats (Crum et al. in 2016). This approach better predicted the overall *in vivo* performance of fenofibrate-loaded LBFs than the classical *in vitro* lipolysis model (Crum et al., 2016), but would not be suitable to act as a rapid screening method due the complexity of the experimental setup. Instead of a membrane, O'Dwyer et al. recently described an adapted version of their biphasic setup, which is described in Section 2.1.1, as an alternative approach to account for absorption in an *in vitro* lipolysis model (O'Dwyer et al., 2020a). This setup more reliably predicted *in vivo* exposure for the majority of formulations tested compared to the pH stat, but could be further refined in future studies to incorporate a more gradual gastric to intestinal transition and enable *in situ* quantification of the aqueous phase.

Despite all these efforts to create a reliable *in vitro* setup for predicting *in vivo* LBF performance, there is still incomplete understanding of the effects of various processes contributing to digestion and absorption. One way forward to address this topic could be to turn to *in silico* models like molecular dynamics (MD) simulation. MD simulation is an *in silico* modelling approach that is suitable for studying complex systems, such as LBFs, since it can model the movement of atoms and molecules and has the potential to even model how colloidal structures form and change. Already there have been some studies with simple LBFs that have localized the API in the LBF before and during dispersion, and it also has potential to describe the fate of the API during digestion (Warren et al., 2013; Birru et al., 2017; Larsson et al., 2017; Warren et al., 2021; Guruge et al., 2021). A recent MD study by Kabedev et al. has demonstrated how danazol could be delivered to the membrane by a bile micelle (Kabedev et al., 2021). MD could also be used to describe how the API and excipients interact with each other and with the surrounding environment (Larsson et al., 2017).

Dispersion and/or lipolysis data have been incorporated as input parameters into PBB modelling. Fei et al. acquired solubility and dissolution data of three fenofibrate-loaded LBFs and one lipid suspension of micronized fenofibrate in biorelevant media. By also considering the precipitation and re-dissolution characteristics, they successfully predicted the absorption behavior and human plasma profiles using the STELLA® software. Since the investigated LBFs belonged to class IIIa and IIIb according to the lipid formulation classification system, *in vitro* lipolysis was not performed and implemented for these formulations (Fei et al., 2013). Similarly, O'Shea and co-workers successfully predicted a reduction in the food effect for fenofibrate, when formulated as a lipid dispersion, compared to the commercial micronized formulation, utilising *in vitro* measurements (solubility and dissolution data) with *in silico* PBPK modelling using GastroPlus® (O'Shea et al., 2015). For formulation development of a Simvastatin-loaded LBF, Ćetković et al. used input of *in vitro* dissolution data into GastroPlus® to identify the optimal site in the GI tract for API release. The results indicated that drug bioavailability could be further improved by using pH-controlled API release systems which dissolve in distal parts of the intestine (Ćetković et al., 2018). The same approach was later used to predict absorption of simvastatin-loaded LBFs containing polymethacrylate polymers as carriers for sustained drug-release (Ćetković et al., 2019).

In an alternative *in silico* approach, Stillhart et al. introduced a mathematical nucleation and growth model to describe API precipitation occurring during an *in vitro* lipolysis experiment of a fenofibrate LBF. Experimentally, API precipitation was monitored using in-line Raman spectroscopy. The *in vitro* digestion results were then correlated with the theoretical model using the MATLAB® program (Stillhart et al., 2013). Building on that study, Stillhart et al. characterized three LBFs containing fenofibrate *in vitro* in dispersion and lipolysis experiments, again using in-line Raman spectroscopy to monitor precipitation. Using the acquired *in vitro* data and also referring back to *in vivo* studies previously performed in pigs, a model was built to calculate lipolysis-induced drug supersaturation. In contrast to results using classical *in vitro* lipolysis, the model predicted that the supersaturation ratio of fenofibrate was lower when sink conditions were applied (Stillhart et al., 2014).

2.3. Drug complexes with cyclodextrins (CyDs)

In the pharmaceutical field, CyDs are widely used to improve the oral bioavailability of poorly soluble compounds (Loftsson and Brewster 1996; Davis and Brewster, 2004). For this purpose, a number of chemically modified CyDs have been prepared to improve the inclusion capacity and the physicochemical properties compared to natural CyDs. CyD derivatives such as hydroxypropyl-β-CyD and sulfobutyl ether β-CyD have been widely implemented in pharmaceutical development due to their high solubility in water and low toxicity after both oral and parenteral administration (Järvinen et al., 1995; Stella and Rajewski, 1997; Savolainen et al., 1998; Stella and He, 2008).

2.3.1. Complex characterization

The interaction between the CyD and the included API is critical to the manufacturing process and the robustness of the formulation, as well as to the biopharmaceutical behavior of the formulation. For a full description of all the analytical characterization methods used, the interested reader is referred to specific literature on this topic (*e.g.*, Dodziuk, 2006).

The formation of a 1:1 complex between the API and CyD in solution can be described by the following reaction:

$$D + CyD \rightleftharpoons D\text{-}CyD \quad (1)$$

where D is the API (drug) and D-CyD is the formed 1:1 complex.

The apparent stability constant $K_{1:1}$ describing the equilibrium (1) is defined by:

$$K_{1:1} = \frac{[D - CyD]}{[D]\cdot[CyD]} \quad (2)$$

where [D], [CyD], and [D-CyD] are the equilibrium molar concentrations of the API, CyD, and D-CyD, respectively.

The stoichiometry of the complex is most accurately determined by a

Job's plot using ^{1}H NMR investigation of the shifts in the δ-values as a function of the proportion between the host and the ligand, *i.e.*, the CyD and the API. Job's plots have been applied by multiple researchers in the CyD field (*e.g.*, Ramos Cabrer et al., 2003, 1999). Although the stoichiometry can also be deduced by fitting parameters from isothermal titration calorimetry (ITC) (*e.g.*, Cooper et al., 1998; Holm et al., 2009), phase solubility studies (*e.g.*, Jansook et al., 2015), affinity capillary electrophoresis (ACE) (*e.g.*, Wren and Rowe, 1992; Holm et al., 2007), the Job's plot remains the only direct measurement approach. In some cases, the stoichiometries are not constant throughout the entire phase diagram, as demonstrated for the interaction between γ-CyD and hydrocortisone (Schönbeck et al., 2017), but for most modified CyDs used in pharmaceutical applications 1:1 stoichiometry is observed at all relevant CyD concentrations. The stability/complexation constant(s) can be determined by the same techniques as described for the stoichiometries, but other techniques such as circular dichroism spectroscopy and fluorescence spectroscopy can be applied.

Typical CyD complexes have low binding constants and extremely fast dissociation relaxation times (< 0.1 s) which makes it challenging to measure the apparent dissociation rates. A number of effects have been demonstrated to influence the stability constant, including type of CyD (*e.g.*, Cirri et al., 2006), degree of substitution on the CyD (*e.g.*, Schönbeck et al., 2010; 2011), temperature (*e.g.*, Loftsson and Hreinsdôttir, 2006), buffer type (*e.g.* Perlovich et al., 2003) and concentration (*e.g.*, Samuelsen et al., 2021), ionic strength and concentration (*e.g.*, Samuelsen et al., 2021). The formulator needs to be mindful of such effects to identify the optimum formulation for a given API. If CyDs are added to a formulation to increase the API solubility, the optimum formulation would most likely be obtained if reaction 1 is driven as far as possible to the right-hand side. This can be achieved by choosing a vehicle in which the affinity of the API for the CyD is enhanced, *e.g.*, by adjustment of pH and addition of water-soluble polymers. A more detailed review of formulation considerations for CyDs has been published by Loftsson and Brewster (2012).

To rank the biopharmaceutical performance of CyD formulations, dynamic processes such as liberation rates and absorption rates (permeation) of the API must be considered. The current opinion is that CyD molecules are not absorbed in the intestines to a significant degree (Stella and He, 2008; European Medicines Agency 2017). Thus, the absorption of a CyD complexed API upon oral administration is a relatively complicated process since the API must be freed from the complex to be absorbed. Under this assumption, if the API has a high affinity for the CyD and the formulation contains large amounts of CyDs, API absorption should be reduced. Two papers have reported such a reduction in bioavailability, but only at extremely high CyD excess relative to the amount required to solubilize the API (Westerberg and Wiklund, 2005; Holm et al., 2016). High concentrations of CyD may directly reduce the free fraction of drug but other mechanisms may also be at play, ameliorating the CyD tendency to "hold on" to the API. A variety of lipophilic compounds, originating from ingested meals and GI secretions, may be able to displace drug molecules from the CyD cavity and make the drug available for uptake. In particular, bile salt displacement of API molecules may take place in the duodenum, provided that complexation equilibria favour this (Ono et al., 2002; Ghorab and Adeyeye, 2003; Loftsson, 2012). Most APIs would easily be released from the CyD complex because the molar amount of bile salts is high relative to the molar dose of API, thus facilitating the displacement from the CyD. Reduction of bioavailability due to lack of release from the CyD complex may therefore be less of a risk for CyD formulations.

To mimic absorption processes for CyD-complexed APIs *in vitro*, measurement of permeation rates in diffusion cells through membranes that only allow the free molecules to pass have been investigated. As a natural reflection of the CyD complexation chemistry, the higher the CyD concentration, the higher the apparent solubility and therefore also the lower the permeability – termed the 'Solubility-Permeability Interplay' or the 'Solubility-Permeability trade-off' (Dahan et al., 2010). This effect has been shown with experimental setups using both artificial and cell-based models (Beig et al., 2013) as well as in a rat perfusion model (Fine-Shamir et al., 2017). The solubility-permeability interplay is a phenomenon observed for all classes of excipients that solubilize molecules, *i.e.*, excipients which form non-absorbable colloidal drug assemblies and similar effects have been reported for various CyDs, polymers (such as PEG) and surfactants (Pluronic F127) in buffers. In all cases, the solubilizing effects of the excipient lead to a lower fraction of free API molecules (Volkova et al., 2021).

Recently, the intestinal uptake of danazol and albendazole in rats from CyD formulations was studied and shown to be dissolution rate limited (Aihara et al., 2021). By contrast, permeation through a PAMPA artificial membrane using the same CyD complexes in buffers revealed that danazol transport was rate limited by diffusion (*i.e.*, dependant on the thickness of the unstirred water layer (UWL) as a function of stirring rate), whereas albendazole transport was limited by the permeation across the PAMPA membrane. Differences in the rate-limiting processes of the different models indicate that a discrepancy in the effect of HP-β-CyD *in vitro* and *in vivo* is to be expected (Aihara et al., 2021). As mentioned above, not only release by dilution, but also by competitive association reactions may enhance the release of the complexed drug molecules and thus improve absorption. However, an *in vitro* permeation study comparing permeation in the presence and absence of bile salts (Eriksen et al., 2022) suggested that the simultaneous presence of the two species (free bile salt and micelles) in CyD solutions can either increase the free fraction of the drug and thus permeability or, depending on stability constants and exchange rates, reduce permeability. Such *in vitro* studies, combined with determination of the chemical equilibria involved, may give more insight towards a mechanistic understanding of the biopharmaceutics of CyD formulations.

The effect of having a mucus layer on the intestinal epithelium on CyD based formulation performance has also been investigated. Several *in vitro* studies have evaluated the effect of mucus on permeability of APIs and the combined effect with CyDs. However, the results depend strongly on CyD concentrations, and on colloids from simulated intestinal fluids as compared to plain buffers (Stappaerts et al., 2018). Active transport of the API may also influence the CyD complex permeability equation, since there are recent signs that β-CyDs may be able to induce a modest inhibition of the P-gp transporters (Bajaj et al., 2021).

In recent reviews, computer-based docking experiments and, in particular, MD simulations have been touted as a very strong tool to investigate the interactions in CyD host–guest complexes (Mazurek et al., 2021; Schmidt and Barner-Kowollik, 2017; Abdolmaleki et al., 2017). As the applied methods become increasingly sophisticated, accuracy relative to the experimental results is improving. The versatility of MD simulations enables all kinds of complexes to be studied, including both native and substituted CyDs, and a wide range of APIs have been modelled using MD simulations to provide a better molecular understanding of the molecular interactions involved (Schönbeck et al., 2014; Tidemand et al., 2014).

Khuntawee et al. (2015) investigated the molecular interaction of β-CyD with a lipid bilayer using MD simulations. They used MD simulation to depict the lipid bilayer and β-CyD for six microseconds and their results demonstrated that β-CyDs diffuse passively into the lipid bilayer. These data are in line with the general knowledge in the field that β-CyDs extract cholesterol from the cellular membrane (Khuntawee et al., 2015). The application of MD simulations for further understanding of CyD-based formulations and their biopharmaceutical behaviors therefore seems to have huge potential. In addition, given the system's high suitability for modelling, it may also be possible to model the interaction with the intestinal epithelium in presence of mucus layer to bring more clarity on that part of the absorption step. Dahan et al. (2010) defined a model to estimate the fraction absorbed from a CyD solution based upon an *in vitro* permeation study in Caco-2 cells. The model relied on measurement of the permeation and the stability constant of the interaction between the CyD and the drug molecule, which

was then transcribed into a predicted bioavailability.

Taupitz et al. (2013) evaluated three novel CyD derivatives and their binary and ternary complexes with Soluplus® in terms of improving solubility and dissolution and inhibiting precipitation of itraconazole. These ternary complexes were evaluated by PBB modelling to define which formulation should work best. Christodoulou et al. (2015) developed a pharmacokinetic model based on *in vivo* data in mice, which was applied in a PBB model to evaluate tissue distribution. Sun et al. (2018) investigated the permeability of progesterone as a function of CyD concentration and used the results as input parameters in a PBB model, which was subsequently shown to align with an *in vivo* rat study. Wang and Ouyang (2021) used the input from the study by Dahan et al. (2010) to construct a PBB model to predict potential overdosing of CyD, which can be considered as an extension of the model described by Sun et al. (2018). While the modelling work of CyD based formulation described in the literature is relatively limited so far, the few studies available demonstrate that PBB modelling may become a useful tool for CyD formulation optimization work when more experimental data become available. Development of an excipient-interaction model for CyDs into PBB modelling is described in Section 3.2.

2.4. Co-crystals and salts

The solid form of an API can have a strong impact on its solubility in the intestine. Co-crystal and salt forms are investigated to enhance solubility of poorly water-soluble drugs (Roy et al., 2011; Bavishi and Borkhataria, 2016; Serajuddin et al., 2007). Both co-crystals and salts are multicomponent single-phase materials, but they differ with respect to their components and the interactions between them (Karagianni et al., 2018; Serajuddin et al., 2007). While co-crystals are usually formed by neutral molecules linked by weak intermolecular interactions, salts are constituted with metal ions or charged organic molecules with acidic or basic functionalities and are held together by ionic interactions (Aakeröy et al., 2007). There are numerous examples in the literature of co-crystals and salts that have been developed to increase drug solubility (Divya et al., 2021; Smith et al., 2011). For example, Kuminek et al. reported a 16-fold increase in the solubility of posaconazole in FaSSIF by forming a posaconazole:4-aminobenzoic acid co-crystal (Kuminek et al., 2019).

Various *in vitro* tests have been used to help assess the enhancement of API absorption by co-crystal or salt formation (Tomaszewska et al., 2013). The μDiss-Profiler™ (Pion Inc.) is a UV fibre optic-based *in vitro* model which enables *in situ* determination of concentration and thus assessment of supersaturation effects (Ando et al., 2012). Martin et al. (2013) demonstrated significant improvement in the solubility of ketoconazole when presented either as the oxalate salt (53-fold) or as an adipic acid co-crystal (75-fold) using the μDISS. The most significant advantage of the μDISS for early development purposes is its small-scale setup, enabling tests to be performed using a much smaller amount of API compared to standard dissolution equipment.

Shake-flask methods are widely used for investigating the solubility of APIs (Vertzoni et al., 2022). Recently, Vasilev et al. (2021) evaluated the solubility and dissolution improvement of itraconazole using the itraconazole 4-aminobenzoic acid and itraconazole 4-hydroxybenzamide co-crystal forms using the shake-flask method. The itraconazole 4-aminobenzoic acid and the itraconazole 4-hydroxybenzamide co-crystals exhibited a 225-fold and a 64-fold increase in solubility, respectively, compared to the free base. However, low thermodynamic stability of these cocrystals indicated a propensity to convert to less soluble forms of the drug during dissolution. Therefore, powder dissolution experiments testing both co-crystals and the API were conducted using a shaking flask method at 37 °C, with aliquots withdrawn at specific time intervals over 360 min. The maximum concentration for the co-crystals showed a 50-fold increase compared to the thermodynamic solubility of the drug, before the drug started to precipitate after approx. 120 min (Vasilev et al., 2021). Despite this precipitation, the concentration for the co-crystal formulations remained at least 10 times higher than the thermodynamic solubility of the drug throughout the experiment. Dissolution studies studying meloxicam cocrystals (1:1 meloxicam-salicylic acid co-crystal and 1:1 meloxicam-maleic acid co-crystal) with the shake flask method were conducted by Machado et al. (2020) in different media (dilute HCL at pH 1.6, pH 5.0 acetate buffer, pH 6.5 phosphate buffer and Fed State Simulated Intestinal Fluid (FeSSIF)). In all media, the co-crystals reached at least 3 times higher concentration compared to the free acid, with highest levels of supersaturation observed at lower pHs where the dose: solubility ratio was greatest.

The USP dissolution apparatus II has also been employed to study the impact of co-crystal and salt formation on API dissolution. However, experience with combining *in vitro* data with PBB models is mostly limited to pharmaceutical salts – little has been published in this area for co-crystals. Through an integration of the dissolution behavior in a PBB modelling workspace, it was possible to predict the *in vivo* plasma concentration of raltegravir after administration as the potassium salt (Segregur et al., 2022). In addition, combination of the *in vitro* experiment and the PBB model were also able to bracket the proton-pump inhibitor (PPI) effect reported *in vivo* (Segregur et al., 2022). Whilst there is significant experience with using the USP II dissolution tester, this methodology does not account for the changing environment of the proximal to distal intestine or take the absorption of the drug into account, both of which can have an impact on the dissolution or supersaturation behavior. In addition, there is an ongoing challenge in evaluating the behavior of salt/co-crystal formulations when comparing their performance across *in vitro* setups, due to the influence of hydrodynamics in each setup.

The BioGIT model (described in Section 2.1.2) has also been successfully employed to match observed concentration of diclofenac and its potassium with the measured concentrations in the duodenal contents, after administration of tablet formulations (O'Dwyer et al., 2022). Kesisoglou et al. (2018) combined dissolution results in the BioGIT using hypochlorhydric biorelevant media for a semi-fumarate co-crystal of a developmental compound with PPB modelling via GastroPlus® to successfully simulate its pharmacokinetic profile.

Modelling of pharmaceutical salts requires knowledge of the salt solubility product (K_{sp}) which can be estimated from the experimental pH/solubility profile, including identification of the pH_{max}. Salt formulations drive the creation of solutions that are supersaturated with respect to free API solubility, thus creating a precipitation risk. The stability of the supersaturated solution is critical to *in vivo* performance and can be estimated from appropriate *in vitro* experiments and used to inform PBB modelling. An example covering some of these aspects is provided by Chirumamilla et al. (2021).

2.5. Deep eutectic solvents

DES are a class of eutectic mixtures consisting of two or more components with a eutectic point far below the melting point of the individual components (Abbott et al., 2003). The term 'deep' emphasizes that the eutectic point is significantly lower than the eutectic point of an ideal liquid mixture (Martins et al., 2019). When mixed at a given molar ratio, the two DES components - one hydrogen bond donating and one hydrogen accepting – form a thermodynamically stable state as a liquid state at room temperature (Martins et al., 2019; Wolbert et al., 2019). DES are widely studied in chemical engineering, green chemistry, and material sciences (Palmelund et al., 2021; Liu et al., 2018). However, these mixtures represent virtually uncharted territory in the pharmaceutical sciences (Chakraborty et al., 2021; Hansen et al., 2021; Morrison et al., 2009). Amongst several applications, certain DES have been studied as solubility enhancing solvent mixtures (Lu et al., 2016; Li and Lee 2016; Sut et al., 2017; Faggian et al., 2016; Jeliński et al., 2019; Palmelund et al., 2021) for APIs that are poorly soluble in water and most other conventional solvents. There are different ways of

formulating a DES as an enabling oral drug formulation (Chakraborty et al., 2021; Morrison et al., 2009). Drugs exhibiting pronounced proton acceptor and donor qualities can serve as one of the two components in a DES system; these mixtures are referred to as therapeutic deep eutectic systems (THEDES) (Aroso et al., 2015). Another, more direct way of using DES in oral formulations is to harness such mixtures as solvent vehicles for active pharmaceutical excipients (Gutiérrez et al., 2018; Jeliński et al., 2019; Cysewski et al., 2019).

Although a vast majority of pharmaceutically oriented studies on DES to date have been in the field of vehicle safety and tolerability (Liu et al., 2018; Hayyan et al., 2016; Faggian et al., 2016), a few studies on the *in vitro* release of API-loaded DES can also be found (Zainal-Abidin et al., 2019; Jeliński et al., 2019; Sut et al., 2017). A recent study focused on oral delivery of aprepitant through enabling DES formulations and compared the *in vitro* and *in vivo* data with a corresponding amorphous system and a commercially available nano-crystalline formulation of the API (Palmelund et al., 2021). The study included *in vitro* permeation investigations using the PermeaPlain® plate method, which was complemented with *in vitro* API release data for the various formulations using a dissolution μDISS Profiler™ with *in situ* fibre optic UV monitoring. The amorphous and DES-based formulations showed pronounced supersaturation, with subsequent precipitation *in vitro*. Hydroxypropyl methylcellulose (HPMC) was then added to the DES formulation to hinder precipitation. Although a beneficial effect of polymer addition was clearly observed in the *in vitro* release test, it was not observed in the permeation study. Although both *in vitro* tests suggested superiority of the DES and amorphous formulations compared to the nanocrystalline drug product, the pharmacokinetic study in rats did not show an advantage in API exposure (Palmelund et al., 2021). More biopharmaceutical research with DES formulations is certainly needed (Chakraborty et al., 2021; Martins et al., 2019; Hansen et al., 2021) to identify more appropriate *in vitro* methods for this formulation approach.

2.6. Nanocrystal systems

Conceptually, the ability of using nanosized drug particles as a bioavailability enhancing approach can be described by the Noyes-Whitney/Nernst-Brunner equation (Kesisoglou et al., 2007). The equation describes the rate from the dissolving surface into the fluid, which is dependant on the surface area exposed to the dissolution medium as well as the hydrodynamic conditions, the diffusion coefficient, and the saturated solubility of the compound. Due to the reciprocal relationship between particle radius and total powder surface area, dissolution rates can thereby be increased through particle size reduction. The nanformulation approach has successfully been described to increase the intestinal absorption and bioavailability of several BCS class II drugs (*e.g.*, Jia et al., 2002; Merisko-Liversidge et al., 2003; Sigfridsson et al., 2009). In particular, the use of nanosized particles are relevant for those belonging to the DCS class IIa *i.e.*, compounds with dissolution rate limited absorption (Van Eerdenbrugh et al., 2008). In addition, nanosuspensions are frequently employed for toxicological studies in the earliest phases of drug development. According to the existing scientific literature, poorly soluble substances with high M_W, high T_m values, and a surface energy equivalent to that of a given stabilizer are capable of forming stable nanosuspensions (Lee et al., 2008). Table 4 outlines some oral pharmaceutical products which contain drugs in the nanosized range, effectively demonstrating the usefulness and potential application of this approach in facilitating the oral absorption of new compounds with limited solubility in water and absorption limited by dissolution rate.

Table 4
List of key oral nanosuspensions-based pharmaceutical products (data from Malamatari et al., 2018).

Trade Name	Drug	Approval Year	Final Dosage form
Rapamune®	Rapamycin/Sirolimus	2000	Tablet
Emend®	Aprepitant	2003	Capsules
Tricor®	Fenofibrate	2004	Tablet
Triglide®	Fenofibrate	2005	Tablet
Megace® ES	Megestrol acetate	2005	Liquid Nanosuspension

In vitro dissolution screening must be considered as the primary step in the biopharmaceutical evaluation of micro- and nanocrystal formulations for oral administration. The formulations are intended to first disperse in the stomach, hence dissolution testing in SGF can provide an initial estimation of the enhancement in dissolution rate in the stomach. For gastric insoluble compounds, where dissolution primarily occurs in the intestinal region, or basic compounds where there is a risk of intestinal supersaturation, further *in vitro* testing in simulated intestinal media offers additional insights into expected bioperformance.

Numerous studies in the literature have reported increased *in vitro* dissolution rates for nanosized APIs. When conducting *in vitro* dissolution studies of formulations containing the drug in nanocrystal form, a major challenge relates to the separation of dissolved and undissolved drug. To address this issue, various approaches have been suggested in the literature, such as filtering through smaller pore size filters (*e.g.*, Juenemann et al., 2011; Kalvakuntla et al., 2016; Liu et al., 2012; Shariare et al., 2019; Thakkar et al., 2011; Xia et al., 2010; Yuan et al., 2016) or (ultra)centrifugation to separate any undissolved API if the dissolution studies are conducted in *e.g.* a USP II setup (*e.g.*, Huang et al., 2013; N. Wang et al., 2021). Alternative usage of dialysis bags in a classical dissolution setup have also been described (Li et al., 2016). These methods help ensure accurate measurement of dissolved API content, where the dissolution media applied would follow the classical recommendations used to evaluate a conventional dosage form *i.e.*, primarily biorelevant media.

Studies have shown that the aqueous boundary layer (ABL) in the intestinal lumen, located near the enterocytes, is not typically the primary barrier for the absorption of small dissolved drug molecules, regardless of their BCS class, under normal physiological conditions (Amidon et al., 1980; Fagerholm and Lennernäs, 1995; Levitt et al., 1992, 1987). However, when an orally administered nanosuspension is present, a pseudo steady-state scenario is created in the intestine. The concentration of free API monomers in the intestinal lumen reaches or closely approaches a saturation stage, due to the rapid dissolution rate induced by the particle size, even if epithelial permeation is fast. In this situation, the diffusion across the ABL, including the mucus layer, may become the rate-limiting step for intestinal absorption. The formation of nanoparticles or drug monomers partitioned into micelles (formed by bile acids and digestive products) can enhance the transport of the API across the ABL, potentially increasing the absorption rate (Roos et al., 2017). Nanocrystals, due to their increased diffusivity compared to microcrystals, are expected to cross the ABL more easily, leading to a higher concentration of API in close proximity to the enterocytes. This higher concentration creates a greater driving force for absorption (Sugano, 2010). A simple absorption model with an artificial membrane may in principle partly mimic this element of nanosuspensions. Studies with dissolution linked to the Caco-2 cell model (Buch et al., 2010), permeation bags (Hens et al., 2015), or *in situ* permeation studies in rats (Roos et al., 2018) have been conducted to investigate the phenomena and improve the predictability of the *in vitro* studies for the *in vivo* performance.

PBB models for micro and nano crystals consider crucial factors such as particle size and distribution. These models aid researchers in understanding whether it is advantageous to pursue particle size reduction and, if so, to what degree it can enhance bioavailability. Commercial software for PBB modelling all have a particle size module that can be used to evaluate the impact of particle size on the bioperformance (*e.g.*, Sjögren et al., 2013; Willmann et al., 2010). More analytical based models do also exist *e.g.*, by deconvolution of *in vivo* data fitting it to the *in vitro* dissolution profile (*e.g.*, Yao et al., 2022) or mathematical based models to calculate the mean particle size that may achieve full

absorption of the compound dosed (*e.g.*, Butler and Dressman, 2010; Hintz and Johnson, 1989; Johnson, 2012). With current scientific insights, the absorption of classical small molecules is methodologically well understood. Modelling of the absorption of nanosized compounds based upon biorelevant solubility and dissolution rate should therefore in general provide sufficient strength in the analysis of the approach, while there would only be a very limited need for non-clinical *in vivo* studies if any during the development of a micro- and nanosized API containing conventional oral formulation.

3. Computational tools to inform PBB modelling of enabling drug products

3.1. Computational tools for predicting biopharmaceutics parameters that are relevant to enabling drug formulations

3.1.1. GastroPlus® tools

The ADMET Predictor® predicts salt solubility factor and supersaturation. The tendency of a compound to supersaturate is defined by a supersaturation ratio (SSR), which corresponds to the ratio of its kinetic solubility (*i.e.*, concentration at the time when a precipitation process is starting) over its intrinsic solubility (*i.e.*, the thermodynamic solubility of the unionized form). Based on this supersaturation model, which was developed from data for 131 molecules, a compound tends to supersaturate when SSR is equal or higher than 1.3. While there is little information on the performance of this model in the public literature, Simulations Plus reports good results based on a small set of test molecules. The current version of ADMET Predictor® does not include the estimation of amorphous and lipid solubility, and K_{sp}, which points to some limitations with respect to parameters that are relevant for predicting the biopharmaceutical behavior of enabling formulations. However, GastroPlus® offers the possibility to include solubility values of different polymorphic forms, which is an alternative approach for using *in vitro* solubility data as input value to the PBB model. The solubility values can be attributed either to the dosed form or to the solid state formed upon *in vivo* precipitation.

The precipitation rate from supersaturated solutions or suspensions can be modelled using either the first order precipitation model or the mechanistic precipitation model. The first order precipitation rate is defined by the mean precipitation time and the size and size distribution of the resulting precipitate particles. The precipitation rate is largely dependant on the intrinsic propensity to precipitate and may vary largely between substances. Hence, this value is often fitted from *in vivo* data in GastroPlus® (*e.g.*, Hens and Bolger, 2019) or fitted from *in vitro* precipitation profiles using DDDPlus® (*e.g.*, Kesharwani and Ibrahim, 2023). GastroPlus® further allows the incorporation of pH-dependant precipitation times, and thus the consideration of gastrointestinal pH and/or excipients effects. The mechanistic precipitation model builds on the classical nucleation theory and currently assumes a homogeneous type of nucleation (Lindfors et al., 2008; Sugano, 2009). In general, the prediction of *in vivo* precipitation remains a significant challenge and has been identified as a current gap in the prospective prediction of low solubility compounds which undergo supersaturation during gastrointestinal transit.

The release or dissolution rate from an enabling formulation may deviate from the dissolution behavior of a conventional formulation. In order to capture the specific dissolution behavior of the formulation and generate a robust mechanistic understanding of the formulation performance, the biopredictive *in vitro* dissolution data can be modelled in DDDPlus using various dissolution models, including the Johnson dissolution model, Nernst-Brunner equation, intrinsic dissolution, as well as the z-factor model. Of particular importance for modelling *in vitro* dissolution data of enabling formulations in DDDPlus® is the possibility to select various apparatus types (USP dissolution apparatus with basket, paddle, or flow-through cell, PION μDiss profiler, the Artificial Stomach and Duodenum) as well as dissolution methods (biphasic dissolution, membrane dissolution), which expands the complexity of simulated *in vitro* conditions. GastroPlus® offers several options to simulate the *in vivo* dissolution rate. These models include the Johnson dissolution model, the Wang-Flanagan model, and the z-factor model. In addition, *in vitro* dissolution data may be used as *in vivo* dissolution profiles in GastroPlus® either via the use of a Weibull function or direct input into the model. Finally, the CyD effect on the API solubility can be considered by specifying the CyD dose in the formulation, its molecular weight as well as the association constant. This is currently possible only for immediate-release dosage forms.

3.1.2. Simcyp™ tools

At present, the broad Simcyp™ strategy is to estimate or confirm required PBB model parameters from the mechanistic modelling of appropriate *in vitro* experiments. Currently, tools are available in SIVA and the Simcyp™ Simulator to consider amorphous solubility, salt K_{sp}, binding to CyD, binding to exogenous micellising surfactant (in addition to endogenous bile salt micelles), and various models for handling supersaturation and precipitation including nucleation.

Within the model supersaturation can be created in various ways. Where an *in vitro* dissolution profile is used as direct input to the model this can drive supersaturation because dissolution rate is taken directly from the *in vitro* profile without regard to local conditions in the GI tract. In this scenario precipitation can only be modelled if it is captured in the input dissolution profile. This mode of input assumes that dissolution is the same regardless of where it occurs in the GI tract and between subjects. Thus, where possible more mechanistic approaches are preferred and available. In these cases, supersaturation can arise due to change in the physiological environment *viz.* pH change, fluid volume change, and reduction in bile salt (or added solubilising excipient) concentration. Within this framework the preferred approach is to parameterize the dissolution and solubility models from the modelling of *in vitro* experiments. Then having gained confidence in the models and their parameters apply them to the *in vivo* modelling. A mechanistic model has the advantage that it is sensitive to physiological parameters which have regional, interindividual and in many cases also interoccasion variability. For fine particle formulations, a diffusion layer model (DLM) based on the Wang and Flanagan approach is implemented which in the Simcyp™ implementation can be used to drive supersaturation even without conditions change (Eq. (3)).

$$DR(t) = \sum_{SS2}^{SS1} \sum_{NBINs}^{i=1} -N_i S_{DR} \frac{D_{eff}(t)}{h_{eff,i}(t)} 4\pi a_i(t) \left(a_i(t) + h_{eff,i}(t)\right) \left(S_{surface}(t) - C_{bulk}(t)\right) \tag{3}$$

where *DR(t)* is dissolution rate at time *t* summed across all *NBINS* (particle size bins) and two different solid states (SS1 and SS2) should for example two different polymorphs be dissolving simultaneously; further description is given in Chirumamilla et al. (2021). $S_{surface}$ is the API solubility at the dissolving particle surface (microenvironment) and is the key to creating supersaturation of API in bulk solution. As discussed below for salts, the microenvironment pH may be significantly different and favourable to salt dissolution and thus salt solubility at the dissolving surface ($S_{surface}$) is elevated. Thus, if the maximal extent of supersaturation before precipitation occurs is established from suitable *in vitro* experiments then this approach can be used to drive supersaturation. The dual solid-state models can also be used to drive supersaturation with respect to crystalline API driven by dissolution of an amorphous formulation, whereby SS1 may be the amorphous form and SS2 the crystalline form each with their respective SS-specific solubility. The model can simultaneously dissolve the amorphous API while precipitating to the crystalline form depending upon conditions and model parameters.

Enabling formulations can (a) drive the creation of dissolved unbound concentrations of API above equilibrium solubility or (b) provide reservoirs of solubilized drug bound to a facilitator molecule such as

CyD, various polymers, or lipids. In the first case, ASDs and salts can be handled via the dual solid -state model structure noted above albeit that for ASDs mechanism is poorly understood and where the dissolution is not API controlled empirical functions are used (*e.g.*, Arora et al., 2020). In the second case, tools for handling inclusion complexes with CyD (and exogenous micelle forming surfactants such as SDS or various Tweens) are available within Simcyp™ (see Section 3.2 and Chen et al., 2020; Hoch et al., 2022) and are provided in SIVA version 5. In these cases, the binding is considered reversible and is treated as additional solubilization terms; where such binding occurs, there is potentially a solubility-permeability interplay which can be modelled (*e.g.*, Chen et al., 2020). However, LBFs are not yet handled mechanistically within SIVA or the Simulator and, thus, can be considered a gap in the modelling tools.

Where true supersaturation is created, the risk for precipitation needs to be considered and the task becomes one of identifying a critical unbound concentration of drug at which precipitation may commence (induction times are considered below) and the rate and extent of precipitation. Again, these parameters can be obtained from the modelling of *in vitro* experiments such as dynamic transfer experiments which to an extent mimic the dynamic nature of the fluid flows *in vivo* (Pathak et al., 2017, 2019; Hens et al., 2017).

With respect to delayed precipitation, linked to the concept of induction times, this commonly arises where an amorphous 'solubility' limit is reached, driven by an enabling formulation. At this limit LLPS may occur and nucleation may initiate within the LLPS droplets (Erdemir et al., 2009). Amorphous 'solubility' can be defined in different ways but such a discussion is beyond the scope of this commentary and the reader is referred to other sources (*e.g.*, Taylor and Zhang, 2016; Vertzoni et al., 2022). Amorphous solubility is a critical parameter within PBB models, since it defines the maximum unbound, unionized, dispersed phase concentration (equivalent to solution activity at low concentrations) driving permeation of the gut wall (Indulkar et al., 2016) and thus the absorption rate. With respect to gaps in the PBB modelling tools, which relates to a lack of mechanistic understanding, nucleation within droplets created by LLPS is not yet handled by the models.

The Simcyp™ tools include a mechanistic nucleation and growth model based upon classical nucleation theory which, coupled to a particle population balance model, has been successfully applied to the modelling of dipyridamole precipitation *in vitro* and within a PBB model (Nimavardi et al., 2021). While further work is required to demonstrate the wider applicability of this model, the dipyridamole study suggests that it may be able to capture induction times. The models are able to handle two different solid states simultaneously which means, for example, that a formulation containing amorphous API can precipitate to a crystalline form within the models or *vice versa*.

A further gap in the modelling tools relates to the use of nucleation and precipitation inhibitors within enabling formulations, the mechanisms for which are still not well understood. Thus, mechanistic models for these interactions are not available in commercial PPB modelling tools to our knowledge. For marketed ASD formulations, such as Norvir® or venetoclax, there appears to be sufficient precipitation inhibitor present in the formulation to effectively stabilize the API at its amorphous 'solubility' limit throughout the absorption phase in the GI tract and this assumption has been successfully applied to building predictive PBB models (*e.g.*, Arora et al., 2020; Emami Riedmaier et al., 2018). Thus, one benefit of developing mechanistic models for such inhibitors may be in helping to define the amount of precipitation inhibitor required.

A further well-established enabling approach is formulation of an API as a salt. Due to their ionic nature and the creation of enhanced API solubility at the dissolving surface (via microenvironment pH effects), salts tend to rapidly dissolve and drive supersaturated concentrations of API with respect to the free form equilibrium solubility. The dual solid-state capabilities with the model mean the salt and free form can be explicitly handled at the same time. Thus, the critical factors are the extent of supersaturation created and the stability of this solution in relation to *in vivo* absorption time frames. The required models for all these factors are available within the Simcyp™ Simulator and the difficulty lies in parameterising the models correctly. This requires appropriate *in vitro* experiments and the mechanistic modelling tools to capture key parameters. Many of these tools are already available in the SIVA toolkit v5. An example of the application of a K_{sp}-based salt model with surface enhanced solubility driving supersaturation is given in Chirumamilla et al. (2021). A knowledge gap with respect to the salt models is the maximal extent of supersaturation that can be created via salt dissolution alone and the stability of these solutions, but these can be addressed through the modelling of appropriate *in vitro* experiments.

3.1.3. GI-Sim tools

In GI-Sim, there are multiple functions that are connected to handling enabling formulations. The amorphous solubility of a drug can be calculated by taking advantage of existing data obtained from Differential Scanning Calorimetry or light scattering experiments (Lindfors et al., 2006). The amorphous solubility determined by these methods has also been used for predicting the *in vitro* dissolution of felodipine amorphous suspensions with good predictability (Lindfors et al., 2007). There is also a possibility to get an estimation through supersaturation experiments (Plum et al., 2017). If experimental data does not exist, a potential way of estimating the input parameter needed is through pure computational methods such as, for example, Monte Carlo free energy simulations, which has been shown to estimate the amorphous solubility within an order of magnitude (Lüder et al., 2009). All the examples above assume that no additional complicating factor is involved once the particles are dissolved. Since an unstable system creating a supersaturated state in the surrounding solution is known to cause precipitation, this has also been included in GI-Sim through the use of classical nucleation theory and particle growth (Lindfors et al., 2008). Hence, all systems such as amorphous formulations and salts have the option in GI-Sim of not only growing existing particles under less favourable dissolution conditions in the GI tract, but also to form new particles with other physical and chemical properties compared to that in the original formulation (*i.e.*, more poorly soluble with separate particle size distribution).

The dissolution of salts is currently simulated in GI-Sim through the same process as a neutral form, with the solubility of the salt as the driving force for the dissolution. *Ksp* is calculated from the solubility at a reference pH. The solubility is inputted directly (thermodynamic value) or as the dissolution ratio relative to the crystal of a net neutral drug. The assumption is that this ratio equals the ratio in solubility. Knowing the solubility of the net neutral drug, the salt solubility is obtained and subsequently *Ksp*. When *Ksp* has been calculated, the salt solubility for various pH values can be calculated. Supersaturation in the GI tract and precipitation to the neutral form can occur in the model. However, work is ongoing, partially through generation of data in the InPharma project, to improve the dissolution model of salts through incorporation of effects directly related to ionic concentrations, dissociation of salt, supersaturation and precipitation surrounding the salt particles.

In media like human intestinal fluids and FaSSIF, there are micelles

present. In GI-Sim, the partitioning to micelles is calculated by comparing the solubility in the micelle containing media with the solubility in the corresponding buffer. Based on the partitioning, the solubility of compounds in media with different micellar volume fractions can be calculated. This also holds for amorphous and salt particles. There is no means presently in GI-Sim to consider the wetting effect that lipids may have on dissolution or lymphatic uptake due to lipids triggering chylomicron formation and incorporation of drug into chylomicrons. The micelles both contribute to providing a sink for dissolution, but they also aid the transport of dissolved drug across the UWL adjacent to the epithelial cells (Sjögren et al., 2013). If the concentration of dissolved drug is lower than the water solubility, then the absorption will decrease due to decreased driving force for absorption.

Irrespective of the type of enabling formulation, GI-Sim describes dissolution as drug diffusion through a stagnant layer surrounding a monodisperse or polydisperse particle. Simulations of dissolution of enabling formulations based on salts, unstable or amorphous forms that deviates from theoretical dissolution based on API particle size are usually handled by using a fitted apparent product particle size distribution. For salts, this will be challenging due to the uncertainty in determining solubility driving the process as precipitation is occurring simultaneously to the dissolution process. Direct input of dissolution data or input through descriptive models such as a Weibull function describing dissolution into the model is also possible, but with the drawback of not being able to utilize local effects of pH and volumes in the GI-tract. More work needs to be done in this area to validate the usefulness of these approaches.

3.1.4. Open systems pharmacology (PK-Sim®/MoBi®) tools

Open Systems Pharmacology (OSP) provides an open source and open access software suite for PBPK and general quantitative systems pharmacology with the dedicated PBPK platform PK-Sim® and the generic modelling platform MoBi® (Lippert et al., 2019). Similar to the concept used in other platforms outlined above, *in vitro* experimental data are at the core of the OSP PBB modelling approach. The versatility and flexibility of MoBi® allows the user to mimic any *in vitro* set-up and estimate the relevant parameters of a dissolution model, which can then be transferred and integrated into PK-Sim®. The available OSP *in vitro-in vivo* translational workflow is applicable to crystalline formulations, yet can serve as a blueprint for enabling drug products (Open Systems Pharmacology, 2021). The workflow features multiple iterative steps to (mechanistically) model the dissolution by using various API and formulation properties. Usually, the particle size distribution is fitted to respective measurements during the first step of this workflow. The API properties (*e.g.*, pKa and thermodynamic solubility) provide then the input for the next step in MoBi®, where the *in vitro* dissolution experiments are set up *in silico*, and unknown dissolution parameters are estimated. Corresponding results of these steps are transferred to PK-Sim®.

For enabling drug products (*e.g.*, ASDs or salts) that potentially create supersaturated solutions, the thermodynamic solubility can be adjusted to the formulation specific solubility obtained from *in vitro* experiments. Dissolution parameters are then optimized using biorelevant dissolution experiments. This approach has previously been used when no precipitation is observed during transfer or 2-stage dissolution assays (Mitra et al., 2016; Emami Riedmaier et al., 2018) and is in accordance with the workflow proposed by the IQ consortium (Aburub et al., 2022).

When precipitation is observed *in vitro*, a first-order precipitation rate can be added to the dissolution model and fitted to the *in vitro* data. However, successful translation using this approach relies on the ability of the *in vitro* assay to accurately mimic the processes occurring *in vivo*. More mechanistic models (*e.g.*, classical nucleation theory) might be needed to capture the prolonged period of nucleation, precipitation, and crystallization occurring *in vivo* and improve the translatability of *in vitro* experiments. These models can be implemented and tested in MoBi®. As part of InPharma, (semi)mechanistic models for drug dissolution and precipitation will be investigated further and will be made available to the scientific community on OSP GitHub to facilitate collaboration and exchange.

3.2. New approaches to computationally account for API – excipient interactions

Any modelling of excipient effects is preferably embedded in a structured formulation development approach (Kuentz et al., 2021). The rDCS gives early insights into drug absorption hurdles and further guidance to formulators may come from an initial PBB model by making use of a parameter sensitivity analysis (Chow et al., 2016; Kuentz 2008). Identification of critical parameter ranges of, for example, drug solubility, dissolution rate, precipitation rate, or permeability, provide targets that can be addressed by formulation technology. A classical modelling approach to formulation/excipient effects would then be to simply change input parameters of a standard PBB model based on *in vitro* data or even calculated results. Much progress has been made already by integrating drug solubility and release data in PBB models. However, for implementation of *in vitro* PRC in PBB models, some care is needed as the *in vivo* relevance of such data in terms of correlation with intraluminal concentrations of API has not yet been consistently demonstrated (Butler et al., 2019; Jamei et al., 2020; Kostewicz et al., 2014). Moreover, this classical approach to account for formulation effects is less appropriate for “bottom-up” modelling as it relies on experimental data (Margolskee et al., 2017). Therefore, it would be preferable, also later in model development, to consider excipient effects explicitly. This would mean adding or, at least, modifying equations in today’s PBB modelling software packages. A first level of complexity would be to account for an API binding constant to any given excipient and at a later stage, models could be further expanded. The added model complexity would be needed, for example, for lipid-based excipients that undergo digestion in the GI tract (Buyukozturk et al., 2013; Stillhart et al., 2013) or where

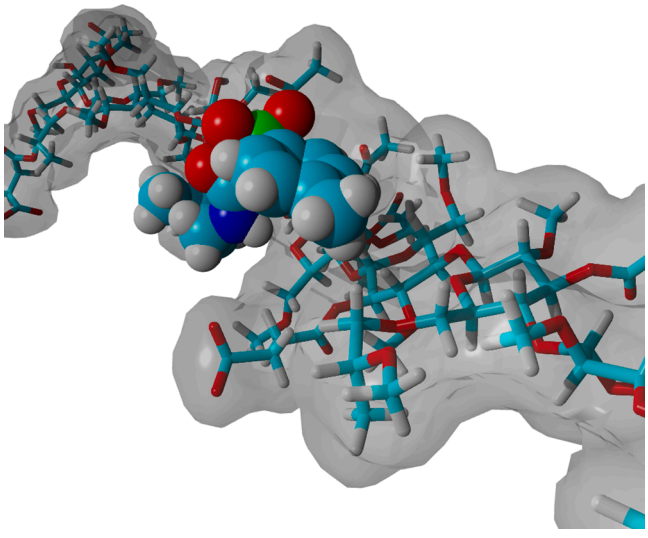

Fig. 2. Result of a simulated drug-polymer interaction [molecular docking in the YASARA software (Krieger and Vriend, 2014)] is shown for tolbutamide and HPMC acetate succinate.

there is competition for binding from, for example, bile components.

At the first level for explicitly accounting for interactions with excipients, different strategies for obtaining API-excipient binding constants and integrating them into PBB models can be taken. An attractive approach is to rely on theoretical calculations or molecular simulations. A toolbox of possible algorithms and models for this purpose already exists (Methat et al., 2019; W. Wang et al., 2021; Kuentz and Bergström, 2021). The opportunities and limitations of theory or simulation-based calculations of drug-excipient interactions for PBB modelling is exemplified in Fig. 2, which shows an atomistic simulation of the interaction of a model API, tolbutamide, with a model polymer, HPMC acetate succinate, in water. In this example, the VINA molecular docking algorithm (Trott and Olson, 2010) was used in the software platform YASARA (Krieger and Vriend, 2014). Considering the different docking poses of drug and polymer, it is possible to calculate a binding constant that could be used further in PBB modelling.

A limitation of any atomistic simulation is the computational requirement, which is particularly high for flexible molecular docking or repeated MD simulations. Moreover, there are often different length scales involved in API-excipient interactions such that a polymer which inhibits precipitation may not only interact with drug in the aqueous bulk phase but may also inhibit crystal growth by adhering to particle surfaces. Such multi-scale modelling is even more demanding and currently this means that it is not a practical way to get the needed input values for PBB models in a timely manner. A bottleneck of calculation time is, for now at least, also an issue for any approach combining quantum chemistry with statistical thermodynamics. Interesting in this context is that the Conductor like Screening Model for Real Solvents (COSMO-RS) (Klamt, 2011), which exists in an accelerated version by making use of an existing database of quantum-chemical calculations to estimate charge densities of new molecules by using a fragment-based approach (Loschen and Klamt, 2012). This method was applied recently to predict drug-polymer interactions with respect to optimal drug precipitation inhibition and a good rank correlation with *in vitro* experiments was obtained (Price et al., 2019). These calculations were based on an excess enthalpy between drug and polymer alone so, despite the good level of theory and the significant correlation with *in vitro* data, the predictive power of this calculation approach for luminal behavior remains unknown. Future research will have to show how broadly this binary mixture approach can be used or how it can be advanced by also considering an aqueous phase that would mimic GI fluids.

As an alternative to using theory or molecular simulations, it would be interesting to employ data-driven estimations of drug-excipient interactions. For any quantitative structure property relationship (QSPR), there is a considerable amount of data needed, which must be gathered first. Therefore, a viable start would be to incorporate an excipient-interaction model into PBB modelling for which one or several drug-excipient interaction constants are estimated from simple experiments. This approach, for a monomeric excipient such as a CyD, has been incorporated into the Simcyp™ Simulator whereby 1:1 or 1:2 (drug: excipient) binding is handled separately for both neutral and ionized species of API, thus up to four binding constants can be applied. The "dose" of the excipient is specified and its concentration-time profile in the luminal fluids is simulated under consideration of absorption into enterocytes and the systemic circulation, as appropriate. Binding of the API to the excipient is entirely dynamic, changing according to molar drug and excipient concentrations, and is treated as an additional solubilization term for the drug. Coupled with binding to bile salt micelles, bound fractions in solution can be estimated enabling the calculation of free concentration (ionized or unionized, as required) of API at any given time, which can then be used to drive gut wall permeation. In addition to inclusion complexes with CyD, the models permit the handling of micelle-forming exogenous surfactants, such as SDS, Tweens etc., whereby the binding of the drug to the micelles is handled using partition coefficients. The free concentration can also serve as a reference concentration for other purposes, such as in precipitation (nucleation) models or metabolic action by the microbiota. The original intention of the model was to aid formulators to decide on the optimal "dose" of CyD via the solubility-permeability interplay (*e.g.*, Dahan et al., 2016). However, in a recent complex case study (Chen et al., 2020) the model was applied to test a hypothesis involving an unexpected DDI between fenebrutinib and itraconazole which could not be explained via the anticipated CYP3A4-mediated metabolic interaction. In that study, itraconazole was formulated with hydroxypropyl-β-CyD, and it was hypothesized that fenebrutinib displaced ITZ from the CyD. Following the hypothesis, the free concentration of fenebrutinib in the luminal fluids would be significantly reduced, with a commensurate reduction in absorption rate (free fraction hypothesis). The required drug-CyD binding constants were estimated from modelling of *in vitro* solubility studies over a range of CyD concentrations (Durk et al., 2020). Despite some limitations (the impact on the itraconazole pharmacokinetics of CyD displacement was not investigated, albeit this is now possible in a more recent version of Simcyp™), PBB modelling plus a preclinical (dog) study (Durk et al., 2020) supported the displacement hypothesis. A second example of such displacement interactions involving itraconazole formulated with CyD is given by asciminib (Hoch et al., 2022). Limitations of the current models include the assumption of instantaneous equilibration within the solubilization/free fraction calculations, and possible unexplored binding interactions of API and/or CyD with other endogenous components of the luminal fluids. The model also requires extension, for example to handle the more complex cases of binding of API to polymeric excipients and the impact of multiple excipients, which may also be non-additive.

4. Looking ahead

Despite the significant progress in understanding enabling formulations to overcome dissolution and solubility limitations to API absorption (Table 5), there is a need to move on from a "trial and error" approach to formulation development to developing fully integrated and computationally informed approaches to formulation selection, without relying on animal testing. The InPharma project aims to bridge this gap by tackling both the formulation and evaluation challenges (Table 6). Computational pharmaceutical tools will be developed to guide formulators with the ambition of eliminating empirical approaches to excipient selection. The computational methods being investigated include quantum chemical methods, molecular simulations and machine learning approaches. Their application to different bio-enabling formulations (amorphous, co-amorphous, mesoporous and co-crystal, microemulsions, salts, deep eutectic and co-milled formulations) will be explored, in order to streamline the early phase of formulation development.

To progress animal-free assessment of API formulation performance, evaluations derived from the rDCS will be compared to animal-based predictions of the optimal formulation type for a range of poorly soluble drugs. Innovative physiologically relevant *in vitro* tools for evaluating drug absorption from bio-enabling oral drug formulations (*e.g.*, ASDs and LBFs) in clinically relevant scenarios will be developed. By exploiting these innovative physiologically relevant *in vitro* tools and adapting *in silico* models to evaluate drug absorption from enabling oral drug formulations in clinically relevant scenarios, a fully integrated in vitro-in silico approach for developing enabling API formulations can be achieved.

Table 5
Benefits and limitations of setups proposed for the *in vitro* evaluation of enabling drug products after administration in the fasted state, under conditions of reduced gastric acid secretion and in the fed state.

In vitro Setup	Benefits	Limitations	Hypochlorhydria	Fed State
Biphasic Testing using InForm instrument	• Small scale • In situ analysis • High level of automation • Incorporates the gastric to intestinal transfer • Rapid absorption to mimic intestinal absorption	• Rapid shift from gastric to intestinal conditions • Susceptible to direct transfer of floating particles to the organic phase • Turbidity in the aqueous phase can impact *in situ* quantification	• Gastric phase set to pH 5 (acetate phosphate buffer)	• Not yet developed
D-P μFLUX	• Small scale • Incorporates the gastric to intestinal transfer • Mimics intestinal absorption	• Rapid shift from gastric to intestinal conditions. • Turbidity in the donor compartment can impact *in situ* quantification • Slow permeation through membrane • Absorption can occur during gastric phase	• Gastric phase set to pH 5 (dilute HCl)	• Not yet developed
PermeaLoop™	• Small scale • IVIVR established for Posaconazole drug products • Improved A/V ratio compared to other setups	• Risk of material adsorption on metal surfaces and tubing. • Non-commercial setup	• Not yet developed	• Not yet developed
Permeapad® plate	• Small scale • High sample throughput • Small amount of material required	• Risk of non-specific adsorption to plate surface	• Not yet developed	• Not yet developed
USP apparatus II	• Well known compendial apparatus • Many customizable dissolution parameters • Compatible with transfer tests	• Large medium and material requirement	• Gastric phase consisting of Level II FaSSGFhypoc-phosphates at pH 5 (Van den Abeele et al., 2020) • Use of FaSSGF ARA (acid reducing agent) pH 4 acetate medium and ARA pH 6 maleate medium (Segregur et al., 2022)	• Gastric phase consisting of level II FeSSGF at pH 5 • Intestinal phase consisting of either FeSSIF V1 at pH 5 or FeSSIF V2 at pH 5.9 (Litou et al., 2020)
BioGIT	• Provides information about the dynamic drug behaviour • Keeps conditions (volume, pH, …) in the duodenal compartment stable	• (Kostantini et al., 2023b)	• Gastric phase consisting of Level III FaSSGFhypoc-phosphates at pH 5 (Van den Abeele et al., 2020)	• Not yet developed
TIM-1	• Advanced representation of the GI tract enabling accurate predictions of oral formulation performance.	• Low-throughput • Absorption is mimicked by simple filters • Risk of API adsorbing on the filter.	• Customized medium at pH 6 (PPI condition) (Van den Abeele et al., 2020)	• Fed state setups available
Tiny-TIM	• Simpler in design than the TIM-1, leading to increased throughput	• Low throughput • Absorption is mimicked by simple filters • Risk of API adsorbing on the filter.	• Elevated gastric pH 6 using citrate buffer and gastric start residue (López Mámol et al., 2022)	• Fed high-fat meal and fed low-fat meal setup available (López Mármol et al., 2022)
pH-stat lipolysis model	• Mimic intestinal digestion • Estimation of the extent of digestion • API phase distribution among micellar phases	• Likely an overestimation of *in vivo* precipitation • No mimic of absorption	• Not yet developed	• Not yet developed
HTP lipolysis model	• Mimics intestinal digestion • Small scale • High throughput	• Likely an overestimation of *in vivo* precipitation • No mimic of absorption	• Not yet developed	• Not yet developed
Two stage digestion model	• Mimics gastric and duodenal digestion	• Likely an overestimation of *in vivo* precipitation • No mimic of absorption	• Not yet developed	• Gastric phase set to pH 6 in fed state
Lipolysis-permeation models	• Mimics intestinal digestion and intestinal absorption • Small scale	• Slow permeation through membranes	• Not yet developed	• Not yet developed
μDISS Profiler™	• Small scale • In situ analysis • High throughput	• Vigorous hydrodynamics may enhance precipitation	• Readily adaptable with use of biorelevant media	• Readily adaptable with use of biorelevant media

(*continued on next page*)

Table 5 (*continued*)

In vitro Setup	Benefits	Limitations	Hypochlorhydria	Fed State
Shaking flask	• Simple setup	• Time-consuming measurements • Manual operation required • Vigorous hydrodynamics may enhance precipitation	• Readily adaptable with use of biorelevant media	• Use of FeSSIF in dissolution test of cocrystals (Machado et al., 2020)

Table 6
Summary of the current challenges and the InPharma's solutions.

Current challenges	InPharma solutions
Trial and error/ empirical decision making process regarding the formulation approach	• Developing mechanistic and/or data driven computational modelling approaches to evaluate the feasibility formulating new drugs as enabling formulations (LBFs, co-crystals, ASDs, co-milling, and DES)
Using animal testing in early formulation development	• Application the rDCS approach to inform formulation selection for a range of recently developed drug substances. • Advancing the framework of rDCS Customised Investigations • Comparison of the rDCS-based approach to animal-based formulation selection approaches
Current *in vitro* methods have limitations for evaluating drug absorption of enabling products	• Refining current and developing new *in vitro* methods to improve the mechanistic understanding of the performance of enabling products (ASD, LBF, Salts, co-crystals, DES)
Gap in knowledge on the performance of enabling formulations in the fed state	• Determination of intraluminal drug concentrations after administration of an enabling drug product in fed state • Designing a biorelevant *in vitro* methodology for evaluating the drug disposition in the upper GI lumen after oral administration in the fed state.
Lack of suitable mechanistic PBB models to assess enabling formulations	• Developing mechanistic PBB modelling approaches for enabling formulations • Coupling biorelevant *in vitro* testing with PBPK modelling to simulate bioavailability advantages of the prototype enabling formulations (ASDs, salts, co-crystals)

CRediT authorship contribution statement

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Declaration of Competing Interest

None.

Data availability

As this is a review article, the data can be obtained (if available) from the source papers.

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