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Successful oral delivery of poorly water-soluble drugs both depends on the intraluminal behavior of drugs and of appropriate advanced drug delivery systems

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ABSTRACT

Poorly water-soluble drugs continue to be a problematic, yet important class of pharmaceutical compounds for treatment of a wide range of diseases. Their prevalence in discovery is still high, and their development is usually limited by our lack of a complete understanding of how the complex chemical, physiological and biochemical processes that occur between administration and absorption individually and together impact on bioavailability. This review defines the challenge presented by these drugs, outlines contemporary strategies to solve this challenge, and consequent *in silico* and *in vitro* evaluation of the delivery technologies for poorly water-soluble drugs. The next steps and unmet needs are proposed to present a roadmap for future studies for the field to consider enabling progress in delivery of poorly water-soluble compounds.

1. Introduction

Poorly water-soluble drugs present ongoing challenges with their translation into viable medicinal products. The hurdles to their successful oral delivery are a complex web of physical-chemical, biological, physiological and anatomical factors that act independently and in concert to limit drug bioavailability. The actions of the mechanical and environmental conditions on the initial dose form is reasonably well characterized – disintegration or rupture of dose forms is rather well understood principally from imaging and other studies (Hens et al., 2017a). However, it is the processing of drug after it is unveiled from the dosage form that is incompletely understood. The solid-state characteristics of the drug and transformations between different states in the gastrointestinal environment are not easily assessed in complex dynamic environments. The more recent trends toward amorphous high energy forms of drug presents a problem of unpredictable crystallization with consequences for solubilization and bioavailability. The tendency toward crystallization can be anticipated, however the complex media of the gastrointestinal tract installs a level of uncertainty around this. The response of excipients to the complex digestive environment of the gut through changes in solubility, degradation by lipases, proteases and other enzymes is individual-dependent, and the consequent interaction of dissolving drug with those components is not yet completely predictable. The gut also responds specifically and

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individually to the nature of the excipients, further complicating the gastric phase of delivery (Ladas et al., 1984). In the case of lipid formulations, drug precipitation on dilution and digestion is an ever-present risk. Digestion of the lipids in the formulation by lipases induces self-assembled colloid formation, and we do not yet know the cascade of structures and specific interactions with poorly soluble drugs that can help or hinder bioavailability as a consequence. Even when the drug is completely dissolved in the remnants of the formulation and ready for absorption, biochemical and post absorption factors usually conspire to further limit bioavailability but may in some cases help by promoting *e.g.* lymphatic transport (Porter et al., 2007).

In consideration of this multi-facetted problem, there is a need to approach this problem from an interdisciplinary standpoint. For decades, pieces of this puzzle have been tackled somewhat in isolation by research groups – while great advances have been made in specific areas, there is no globally unifying approach to bring these findings together to present ways to tackle such problematic drugs in a holistic manner. We may never get there, but by bringing together multidisciplinary clusters to work at the interfaces between groups, we provide the best opportunity of learning how to address these multiple barriers through new delivery technologies, diagnostic approaches and analytical advances.

With this principle in mind, the recently formed European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP), funded under COST action CA16205, are aiming to advance the field of intestinal drug absorption through a multidisciplinary internationally collaborative approach. Two of the four major challenges they have defined relate to poorly water-soluble drugs, namely the intraluminal behavior of advanced formulations (usually required for the effective delivery of poorly water-soluble drugs), and the food-drug interface, which is crucial in consideration of the often lipophilic nature of such compounds.

The purpose therefore of this review is to share the current state of knowledge around the issues, approaches and requirements for future developments in the field as seen by this multidisciplinary team. For the team, this enables definition of both a foundation and roadmap to impact across these challenges, a large initial part being to share a common language, terminology and understanding of needs in the field. For the general reader, this review collates current thinking from this diverse group of researchers, united in the goal to advance the delivery field around poorly water-soluble drugs. The review defines the challenge presented by these drugs, outlines contemporary strategies to solve this challenge, consequent compendial and biorelevant *in vitro* evaluation of the delivery technologies in order to limit the usage of animals, before moving onto defining the unmet needs and future directions for the field and for UNGAP to address. The need for consideration of new paradigms is timely with developments in not only materials for drug delivery, but also in new imaging and analytical techniques and facilities that if embraced and addressed by such a multidisciplinary approach can lead to significant gains in overcoming the limitations to formulation and delivery of problematic compounds.

2. Challenging molecules after oral administration and common formulation strategies to overcome these unfavorable compounds characteristics

A large fraction of contemporary drug compounds has physicochemical properties that may result in low chemical stability in the gastrointestinal (GI) tract, poor and/or variable solubility in the fasted and fed state gastrointestinal fluids and eventually poor permeation across the intestinal wall. Most of the small molecules display poor aqueous solubility and it is not uncommon that lead compounds selected during the discovery stage show solubility in the lower μg/mL region. This fact has been attributed to the chemical approaches used, the organizational factor and last but not least, the lipophilic molecular requirements of contemporary targets (Bergström et al., 2016; Keserü and Makara, 2009; Leeson and Springthorpe, 2007; Leeson and St-Gallay, 2011; Vieth and Sutherland, 2006). Indeed, many targets currently under exploration have highly lipophilic endogenous ligands, which is translated to the need for certain lipophilicity in the modulating drug. The impact of molecular properties on solubility, and the role of the fasted and fed state on the resulting solubility, has been explored by different computational tools; the relation between physicochemical properties and solubility is further discussed in Section 2.1. Taken together, poor solubility and permeability are significantly limiting the absorption of contemporary drugs after oral delivery. In the next section, the relationship between molecular properties and solubility/permeability will be described.

2.1. Physicochemical considerations

Yalkowsky and coworkers established the General Solubility Equation (GSE) in 2001. This identifies the strong link between the solid state and the lipophilicity of the compound, and the resulting aqueous solubility (Jain and Yalkowsky, 2001):

$$\log S_0 = 0.5 - 0.01(T_m - 25) - logP \quad (1)$$

where S_0 is the intrinsic solubility, *i.e.* the solubility of the non-ionized (neutral) species, T_m is the melting point (°C) and logP is the partition coefficient between octanol and water. Wassvik et al. used the GSE and hypothetical values for melting point (T_m of 50, 150 and 250 °C) and the lipophilicity (logP of 2, 4 and 6) to separate compounds that are mainly limited by the solid state, from those limited by their poor solvation (Wassvik et al., 2008). Compounds with a strong crystal lattice often show a limited capacity to dissociate from the solid form and these are commonly referred to as 'brick dust' molecules. In a similar way, a logP cut-off of 3 has been put forward as an indicator of significantly reduced interaction with aqueous solvents (Bergström et al., 2016). Compounds with a logP > 3 are commonly referred to as 'grease ball' molecules. It should be noted that, for ionizable compounds, it is the corresponding logD value (at the pH of interest) that should be greater than the logP cut-off value (Fagerberg and Bergström, 2015). In addition to these, there are compounds that display both high logP and high melting point, *i.e.* they are both solid state and solvation limited in the solubility. Computational modelling of several dataset has linked molecular properties to solid-state- *versus* solvation-limited solubility (Bergström et al., 2007; Fagerberg et al., 2010; Wassvik et al., 2008; Zaki et al., 2010). Solvation-limited compounds are lipophilic, relatively large molecules, and lack conjugated systems. In contrast, common features for solid-state-limited compounds are flatness, extended ring structures and high aromaticity. In addition to modelling of solubility in pure water or simple buffers, models to identify solubility in more complex solvents such as intestinal fluids have been developed (Fagerberg et al., 2015). In these studies, size and aromaticity were negatively linked to the solubility, whereas the hydrogen bond capacity (donors and acceptors) was a positive factor for solubility.

It is well-known that lipophilicity is positively linked to permeation. However, there is also a trade-off when it comes to lipophilicity; too lipophilic compounds may strongly favor the lipid-rich environment to an extent at which its permeation across the lipid bilayer becomes limited. Negatively linked properties to permeation are size and polarity, the latter typically being described as polar surface area, or hydrogen bond donors and acceptors (Palm et al., 1997; Veber et al., 2002). The polarity limitation significantly reduces the permeation of small, hydrophilic molecules and these eventually make use of different active transporters or they permeate the intestinal wall *via* the paracellular route. To further improve the permeability of such compounds, chemical modifications may be used to change the physicochemical properties. A common strategy to apply is to develop prodrugs which either block polar groups and increase the lipophilicity of the molecule, and hence increase the transcellular passive diffusion across the enterocytes or couple a handle 'visible' to active transporters (Murakami,

2016; Wang et al., 1999). From a formulation perspective, permeability enhancers may be added to increase the flux through the paracellular route (Anderberg et al., 1993; Lindmark et al., 1995). This is especially relevant for macromolecules, where the size and polarity result in a limited permeation. Also, for such compounds chemical modifications can be used to increase the membrane permeability with the main focus being to reduce the polarity. One successful strategy is to design molecules with capacity to form intramolecular hydrogen bonds. This enables the compound to display the hydrogen bond donors and acceptors to the water phase in the intestine and thereby increasing the water solubility, whereas the molecule shields these functions from the lipid bilayer through internal bond formation when it encounters the lipid environment and thereby increases the permeation through the cellular membrane (Rossi Sebastiano et al., 2018).

2.2. Solubility and dissolution rate enhancement

Insufficient aqueous solubility compared to the dose that needs to be administered (dose number $D_0 > 1$, Biopharmaceutic Classification System (BCS)) is one of the most frequently encountered problems for drug substance formulation. The low equilibrium solubility leads to very slow drug dissolution rate and poor intestinal absorption. The current formulation strategies to overcome these issues can be separated in two major categories: (1) methods that increase the apparent equilibrium drug solubility *and* the dissolution rate, and (2) techniques that increase the dissolution rate and facilitate the formation of metastable supersaturated drug solutions. Of course, sophisticated techniques that combine the properties of (1) and (2) also exist (*e.g.* salts + precipitation inhibitors, some lipid-based formulations). An important aspect of solubility is the distinction between apparent drug solubility (*e.g.* drug solubilized in micelles, liposomes, cyclodextrins *etc.*) and molecularly soluble "free" drug (*i.e.* in supersaturated solutions). The implications of the latter on drug permeability and absorption are discussed in detail in Section 5.4.

In the next paragraphs, the main concept, application scope, advantages and limitations of the techniques that are widely used to enhance drug solubility in the context of oral delivery (pharmaceutical salts) or are emerging as enabling technologies (amorphous solid dispersions, lipid-based formulations) will be shortly described, including example cases where appropriate. The drug solubilization by surfactants will also be briefly outlined, due to the wide spread use of this family of excipients in both standard and advanced formulations. Approaches with limited application in the context of oral delivery (*e.g.* co-solvents, polymeric micelles, liposomes), or which have been recently reviewed (cyclodextrins (Adeoye and Cabral-Marques, 2017) will be omitted from the current discussion). Although particle size reduction techniques increase the dissolution rate, their effect is limited for drugs with very poor equilibrium solubility in water. Hence, they find application only in combination with other solubility enhancement approaches and will not be described separately. The lipid-based drug delivery systems and their central role as enabling formulations for the oral route are presented at the end of the section. The presented technologies may be used as (pre)clinical formulations or intermediate oral products. These are clearly emerging systems for oral delivery of poorly water-soluble drugs.

2.2.1. Salt formation

The solubility and dissolution rate of ionizable drugs can be improved significantly by preparing their respective salts. Due to the simplicity and cost-efficiency of the concept, it has been extensively used both for oral and parenteral delivery (Paulekuhn et al., 2007). The major advantage of the method is that it can provide considerable increase of solubility (often by > 3 orders of magnitude (Elder et al., 2013)) and dissolution rate without the need to chemically modify the drug molecule or to use complex enabling formulations.

In order to have a complete proton transfer and, hence, obtain a salt, a difference of 2–3 units between the pK_a of the counterion and the pK_a of the drug is required (Berry and Steed, 2017). Drug molecules with more than one ionization moiety display more complex behavior, due to the polyprotic and polybasic equilibria (Maurin et al., 2002). In this case, whether the mono- or poly-salts are preferred for development has to be decided depending on their solubility properties, stability, scalability *etc.*

The solubility of the salt can depend significantly on the type of counterion and should be directly linked to parameters such as the crystal lattice free energy and the hydration free energies of the ions (Anderson and Flora, 1996). However, there are still no approaches that allow its accurate prediction. For example, the type of structurally similar organic acids (tartaric, succinic, lactic, acetic) had no significant effect on the solubility of the weak base avitriptan (Serajuddin, 2002), whereas for diclofenac the addition of one OH-group to the *tert*-butylamine counterion increased the salt solubility 4-fold (O'Connor and Corrigan, 2001). Furthermore, using a tertiary amine (2-dimethylaminoethanol) as a diclofenac salt forming agent resulted in a > 80-fold increase of the salt solubility, compared to the *tert*-butylamine salt.

Another important parameter of a salt that has to be considered, especially in the context of oral delivery, is the pH_{max}: the pH value at which the maximum solubility of the drug is obtained. This parameter is critical, as it governs the conversion of the ionized form to its conjugate free base or free acid and, hence, the precipitation behavior in the gastrointestinal tract. The higher the pH_{max} of a basic drug (or the lower, for an acidic drug), the easier the formation of a salt is, and the better its stability to conversion to the non-ionized form. The magnitude of the pH_{max} for a basic drug can be assessed by Bogardus and Blackwood (1979):

$$pH_{\max} = pK_a + \log \frac{S_0}{K_{sp}} \tag{2}$$

where pK_a is the ionization constant of the drug, S_0 is the intrinsic solubility of the non-ionized form of the drug and K_{sp} is the solubility product of the salt. Therefore, for a basic drug, the pH_{max} increases with the increase of the strength of the base (higher pK_a), with the increase of the solubility of the base (S_0) and with the decrease in salt solubility (K_{sp}). A similar equation, which shows that for an acidic drug pH_{max} decreases with the increase of S_0 and with the decrease of pK_a and K_{sp} can also be written.

The solubility product of the salt is important not only because it characterizes its solubility and influences the pH_{max}, but also in relation to the common ion effect that can dramatically reduce salt solubility in biorelevant conditions. In particular, the solubility of hydrochloride salts of basic drugs can be significantly decreased in the stomach, due to the high concentration chloride anions *via* the following equation:

$$S_{ion} = \frac{K_{sp}}{C_{CI}} \tag{3}$$

where the S_{ion} is the solubility of the drug in its ionized form and C_{CI} is the counterion concentration. The latter leads to the counterintuitive effect of decreased solubility when the pH is significantly lower than the pH_{max} for basic drugs. Note that the common ion effect in stomach conditions should be considered not only when hydrochloric acid is used for salt preparation, but also when weaker acids (*e.g.* organic) are used, as these salts could be transformed to the respective hydrochloride salt *in situ* in the stomach. A pronounced effect can also be expected when the solubility of the salt is in the low-millimolar range, *i.e.* for drugs with extremely poor water solubility and low solubility enhancement.

A major issue with the application of salts in oral delivery is their behavior in biorelevant conditions. If the pH_{max} is not in the range of physiological pH values, precipitation can occur. This is usually the case for acidic drugs in the stomach and basic drugs in the intestine. However, the quick redissolution of the precipitate (phase-separated drug) normally facilitates significantly higher oral absorption,

compared to the non-ionized form. The mechanisms that account for the quick redissolution include high surface are of the precipitate (*e.g.* Phenytoin sodium (Dill et al., 1956; Serajuddin and Jarowski, 1993)) and formation of non-equilibrium metastable states with high thermodynamic activity (*e.g.* emulsion of supercooled melt droplets of diclofenac sodium (Stahl and Nakano, 2002) or amorphous gel formation (Serajuddin, 2007)).

During the selection of a pharmaceutical salt for development, a number of additional factors such as solid-state properties (Raumer et al., 2006), stability (Nie et al., 2017; Stephenson et al., 2011) and toxicity (Stahl and Wermuth, 2008) should also be considered in detail. The solubility of the salt and pH_{max} are not always the main determinants in selection, as scalability and stability during processing (*e.g.* deliquescence, amorphization, disproportionation) might prevent manufacturing of the salt and could warrant the selection of a salt with lower solubility, but better stability, or the use of the non-ionized form (Korn and Balbach, 2014).

There are several limitations of the pharmaceutical salts approach, the most important being the requirement for ionizable groups in the drug molecule. The latter can be circumvented in some degree by using the co-crystallization approach, as highlighted by several recent reviews (Elder et al., 2013; Kuminek et al., 2016). Cocrystallization takes advantage of intermolecular interactions (usually of hydrogen bond-type) between the poorly soluble drug and a hydrophilic coformer to produce cocrystals with significantly enhanced drug solubility. Guidelines for cocrystal synthesis and the importance of cocrystal eutectic constants as a tool for prediction of cocrystal behavior in different media (pH, surfactants) are described in the comprehensive review by Kuminek et al. (2016).

Another limitation is that the degree of solubility enhancement might not be sufficient for drugs with extremely poor aqueous solubility (*e.g.* itraconazole), and that the common ion effect would be very pronounced. In these cases, ionic liquids could be prepared (Agatemor et al., 2018): these are low-melting point (T_m < 100 °C) salts that consist of a drug + bulky counterion. They could either be used for oral delivery by themselves (Shamshina et al., 2013), or if the counterion is hydrophobic, the resulting hydrophobic ionic liquid could be combined with lipid-based formulations (Williams et al., 2014a) that will be described later in the section.

Another option is to combine the salt concept with other solubility enhancement approaches, such as the amorphous solid dispersions + precipitation inhibitors, which will be described in the following paragraphs.

2.2.2. Amorphous solid dispersions

One of the modern approaches that provides both dramatic increase in drug dissolution rate and suitable supersaturation conditions is to modify the solid-state properties of the drug. Ideal amorphous solid dispersions (ASD) can be defined as glass solutions of a poorly soluble drug in an amorphous carrier that represent a single-phase amorphous system (van den Mooter, 2012).

The ease of preparation of a drug in amorphous from can be straightforward (good glass formers) or difficult (poor glass formers) (Yu, 2001). In general, molecules that are difficult to arrange in a crystal lattice, have high conformational flexibility and/or have configurational isomers, tend to have small difference in the free energy of the crystal state, compared to the amorphous state and thus are good glass formers. The classical amorphization techniques could be broadly separated in three main categories: (1) mechanical energy input methods, (2) solvent methods and (3) melt methods. The first category includes different type of mills (*e.g.* oscillatory ball milling, fluid energy mill) (Descamps and Willart, 2016) and wet granulation. The second group consists of anti-solvent techniques, lyophilization and spray-drying (Singh and Van den Mooter, 2016). The third group includes melt agglomeration (the drug melt is used as a granulation liquid) and hot-melt extrusion (the drug is melted or/and dissolved in a polymer melt, which is then cooled down and extruded (Sarode et al., 2013)). Each family of techniques has certain advantages and pitfalls. The common milling methods are simple and do not require complex machinery but have lower amorphization efficiency and are less robust. On the other hand, the solvent and melt methods have good scalability and are used in the manufacturing of ASD. Solvent methods allow the formation of ASD at low temperatures, but then face difficulties in eliminating traces of solvent (often toxic) from the final product. On the other hand, melt methods do not have problems with toxicity (if the polymer and/or other excipients are biocompatible), but cannot be used for thermally unstable compounds and the selection of a polymer with high molecular drug solubility is still a challenge. For more detailed description of the ASD manufacturing methods the reader is referred to a recent review article (Vasconcelos et al., 2016).

A major issue in ASD formulations is their innate thermodynamic instability, related to the transition of the amorphous solid to its stable crystal form. One of the key parameters in this context is the glass transition temperature (T_g), which is defined as the critical temperature below which a glassy solid is obtained. Higher T_g is associated with better physical stability during storage and processing. An additional layer of complexity is that the transition of an amorphous solid to crystalline state can pass through a number of crystalline mesophases (Shalaev et al., 2016) with intermediate properties. Therefore, the solid-state properties of the amorphous material during *in vitro* dissolution and *in vivo* experiments, as well as during processing and storage, has to be monitored if mechanistic interpretation of the data is desired. However, the complex character of the different relaxation processes in amorphous systems (time scale span of > 10 orders of magnitude (Hancock and Shamblin, 2001)) has posed a significant challenge. The analytical techniques which allow one to assess the molecular mobility and thus judge the solid state of the drug include calorimetric, spectroscopic and scattering methods. In recent years, significant progress has been made in the use of Raman (Hédoux, 2016) and terahertz spectroscopy (Sibik and Zeitler, 2016), broadband dielectric spectroscopy (Grzybowska et al., 2016) and X-ray diffractometry (Thakral et al., 2016) and the reader is referred to the respective reviews.

The stabilization of the drug in amorphous form can be obtained by using polymers (Ubbink, 2016), mesoporous silica (Mura et al., 2019), or by preparing co-amorphous formulations with a second drug or low molecular weight excipient (Dengale et al., 2015). The polymers have been the most widely used excipients for stabilization of amorphous drugs by forming molecular dispersion with the drug and thereby limiting the mobility of the drug molecules, which, in turn, inhibits crystal growth and nucleation (Liu et al., 2015). Here the T_g of the polymer matrix is of utmost importance and $T_g \geq 50$ °C has been recommended in order to compensate the plasticizing effect of the drug and ambient moisture (Hancock et al., 1995).

The application of the ASD concept, as well as some of the important stability issues are illustrated in the recent study of Knopp et al. (2018), who prepared an ASD of celecoxib with polyvinylpyrrolidone (PVP) by melt quenching. The ASD formulation increased dramatically drug dissolution rate and resulted in considerable supersaturation, as compared to the crystalline drug: the dissolution area under the curve at 4 h (*in vitro* AUC_{0-4h}) of the ASD formulation was 67.2 ± 0.3 mg·min/mL, compared to 12.8 ± 0.3 mg·min/mL for the crystalline drug. This effect translated into a ≈ 3-fold higher *in vivo* exposure in rats: AUC of 294 ± 16 μg·h/mL of the ASD formulation, compared to 105 ± 10 μg·h/mL for the crystalline drug. To study the effect of the drug crystallization, which could occur during storage, the authors varied the fraction of crystallized drug by preparing mixtures of the pure ASD and the crystalline drug at different ratios. Although the *in vivo* AUC decreased linearly with increasing the crystallized celecoxib fraction, the difference between the pure celecoxib ASD (0% crystalline

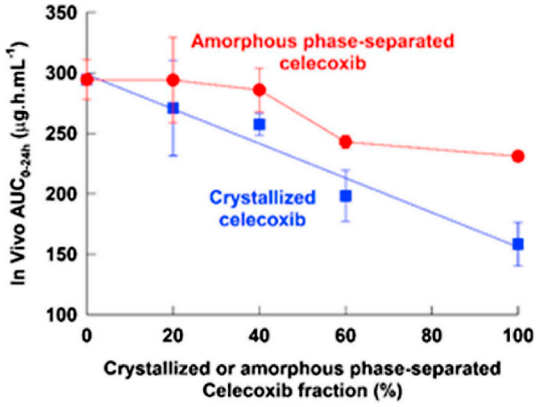

Fig. 1. Relationship between the fraction of crystalline (blue squares) or amorphous phase-separated (red circles) celecoxib (% w/w) in the ASDs and *in vivo* AUC0-24 h ± SEM (n = 3–5). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Reproduced from Knopp et al. (2018).

drug, AUC = 294 ± 16 µg·h/mL) and 40% crystalline celecoxib (AUC = 258 ± 9 µg·h/mL) was not statistically significant, see Fig. 1. Significant decrease in the AUC was observed upon further increase of the crystalline celecoxib to 60%: AUC = 198 ± 21 µg·h/mL. The *in vivo* behavior of the formulations with different content of phase-separated amorphous drug domains showed a similar pattern, see the red circles in Fig. 1: no significant difference in the AUC was observed up to 40% phase-separated amorphous celecoxib fraction, followed by a significant decrease in the AUC at 60% amorphous fraction. Hence, the obtained results suggested that the partial transformation of an amorphous drug to crystalline state or phase-separate amorphous domains will not have significant effect on the *in vivo* exposure.

One should stress, however, that an important aspect of the ASD techniques is the time window of sustained supersaturation, which drives drug absorption and bioavailability. The rate of precipitation and the possible approaches to kinetically trap the system in this thermodynamically unstable state will be discussed in Section 2.3., as well as the supersaturation advantage if amorphous precipitate is formed (Section 2.4).

2.2.3. Solubilization in surfactant micelles

Surfactants are frequently encountered in classical and enabling formulations (*e.g.* lipid-based formulations) and contribute to the drug solubility enhancement in the carrier systems, as well as after application. In addition, the drug is solubilized by the endogenous surfactants (bile salts, phospholipids) present in the intestine. Hence, understanding the main factors and mechanisms governing micellar solubilization is required for successful application of surfactants in the complex enabling formulations used in oral drug delivery.

Drug solubilization occurs above the surfactant critical micelle concentration (CMC), where the surfactant molecules form micelles (Rosen, 2004): colloidal aggregates with heterogeneous microstructure, which contain regions with different polarity. The varying polarity in the micelles facilitates the incorporation of poorly water-soluble drug molecules, which results in solubilization, *viz.* the increase in the apparent aqueous solubility of the drug. The CMC is also directly linked to the stability of the micelles upon dilution in biological fluids: in general, surfactants with low CMC values (CMC ≪ 1 mM, *e.g.* some non-ionic surfactants, amphiphilic polymers) are more stable.

The solubilization of drugs by surfactant micelles in simple aqueous solutions is an extensively studied topic (Bhat et al., 2008; Bodor, 1984; Krishna and Flanagan, 1989; Ong and Manoukian, 1988; Park and Choi, 2006; Stoyanova et al., 2016; Ullah et al., 2014; Vinarov et al., 2018b; Vinarov et al., 2018a; Vinarov et al., 2018d). Significant increase in drug solubilization capacity with increasing surfactant chain length is documented for several surfactant families (alkylsulfates, alkyltrimethylammonium bromides, alcohol ethoxylates, polysorbates) and for drugs with diverse structures, such as steroids (Ong and Manoukian, 1988; Vinarov et al., 2018a; Vinarov et al., 2018d), benzophenones (Vinarov et al., 2018d), benzimidazole (Vinarov et al., 2018b), macrocyclic lactones (Bhat et al., 2008), sesquiterpene lactones (Krishna and Flanagan, 1989) anthranilic and propionic acid derivatives (Stoyanova et al., 2016; Ullah et al., 2014). The effect of surfactant hydrophilic head group depends on the specific drug-surfactant interactions: in the case of electrostatic attraction, the solubility can be increased by several orders of magnitude (as shown for weakly basic (Vinarov et al., 2018b) and acidic drugs (Park and Choi, 2006)), whereas ion-dipole interactions also increase drug solubilization, but to a lesser extent (Vinarov et al., 2018a; Vinarov et al., 2018d).

A major challenge in the context of solubility enhancement *via* surfactant solubilization is the colloidal instability of the drug loaded micelles when they are introduced into a medium containing bile salt. A recent study on the behavior of conventional surfactant micelles in biorelevant media showed that several drugs (fenofibrate, danazol and progesterone) precipitated when ionic surfactant micelles were introduced in bile salts-containing dissolution media, due to formation of mixed micelles with low solubilization capacity. In contrast, polysorbate surfactant micelles did not form mixed micelles and remained stable, see Fig. 2 (Vinarov et al., 2018c). Hence, systematic studies in biorelevant media in the presence of bile salts are required to clarify the role of surfactant micelles in the solubility enhancement techniques for oral delivery.

A recent study differentiates between the effect of diacyl- and monoacyl phospholipids on the solubilization of celecoxib and its

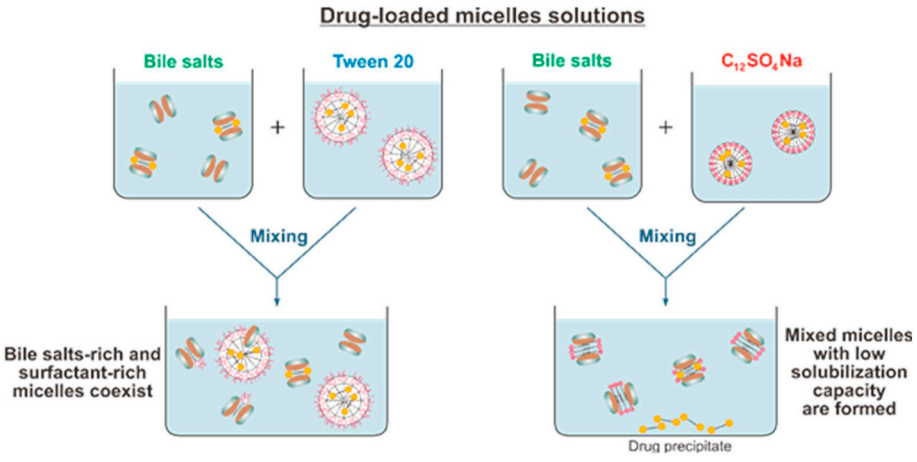

Fig. 2. Illustration of the fate of drug-loaded micelles in biorelevant medium. Drug-loaded micelles of surfactants and bile salts were prepared separately and then mixed at a 1:1 ratio. The solution of Tween 20 + bile remained clear, whereas precipitation was observed in the mixtures of bile salts with ionic surfactants. The coexisting bile salt and Tween 20 micelles were determined by 1H DOSY.
Reproduced from Vinarov et al. (2018c).

Table 1
The lipid formulation classification according to 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

impact on *in vitro* permeability (Jacobsen et al., 2019). The authors showed that the solubility enhancement of the monoacyl system was significantly better, compared to the diacyl. The solubility enhancement was maintained also in presence of bile salts (FaSSIF) for both studied phospholipid types, which indicated that they have good stability in biorelevant media. However, no significant difference was observed in the rate of permeation in a side-by-side setup, which indicated that the process was controlled by the concentration of free drug and not the apparent drug solubility.

2.2.4. Lipid-based formulations

The formulation of drugs in a lipid carrier system composed glycerides, surfactants and co-solvents has attracted significant attention due to the markedly increased drug oral bioavailability (Porter et al., 2007). Apart from the solubility enhancement effect, the mechanism of improved bioavailability of lipid-based formulations (LBF) is attributed also to increased intestinal absorption *via* supersaturation (Gao and Morozowich, 2006) and reduced first-pass effect *via* lymphatic transport (Trevaskis et al., 2008).

LBF are primarily applied for BCS Class II and Class IV drug substances, which are characterized by solubility-limited absorption. The lipid formulation classification system (LFCS) separates LBF in 4 main types, depending on LBF composition, see Table 1 (Pouton, 2006).

Each type is characterized by a set of advantages and drawbacks. For example, the glyceride-rich types 1 and 2 usually have poor drug solvent capacity but are unlikely to lose that solvency upon dispersion in the intestinal fluids, whereas the solvent- and surfactant-rich LBF types III and IV can dissolve higher drug concentrations but suffer from significant phase changes and potential drug precipitation upon dispersion. One aspect of LBF is that a significant fraction of the surfactants used may be digestible, which should be considered during formulation development (Vithani et al., 2017). The different formulation strategies and the materials used are described in detail in several dedicated reviews (Hauss, 2007; Pouton and Porter, 2008).

However, as the oral absorption depends on the intestinal concentration of dissolved drug, another LBF classification related to formulation performance has been proposed (Williams et al., 2014b). The latter groups LBF into four grades (A, B, C and D) where grade A provides the most robust solubility enhancement after dispersion and digestion, whereas drug precipitation is expected with increasing

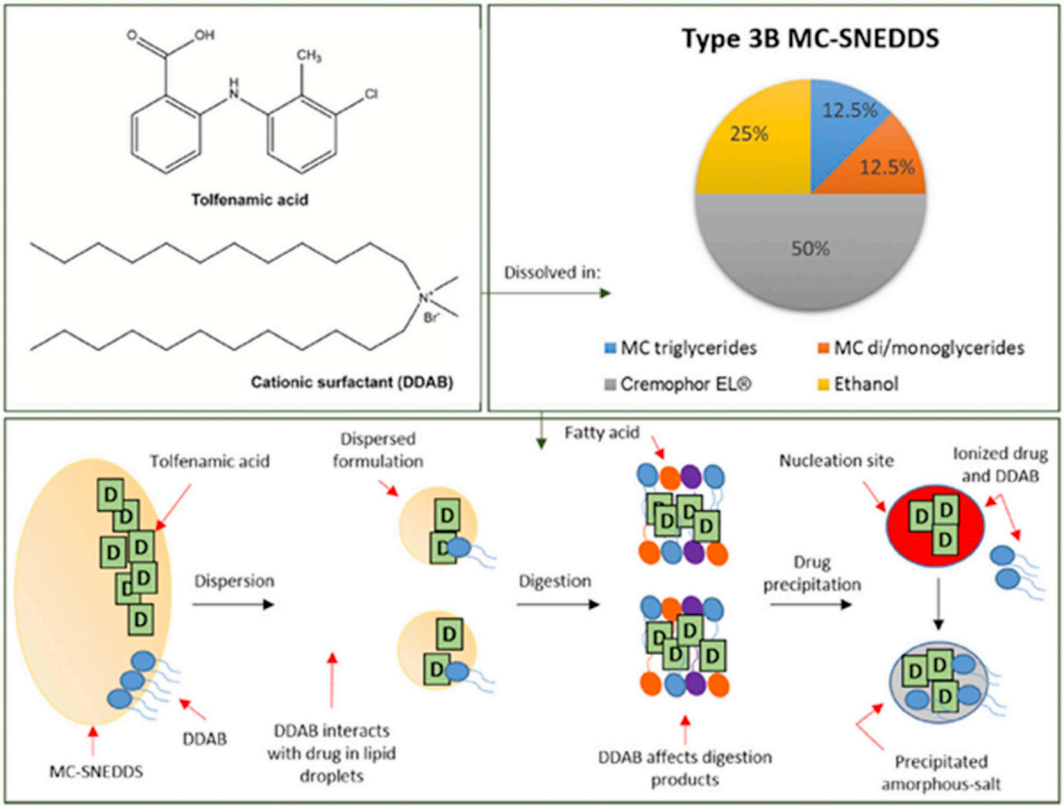

Fig. 3. Top left: Chemical structures of tolfenamic acid and the cationic surfactant didodecyl ammonium bromide, DDAB. Top right: Composition of the model type IIIB MC-SNEDDS used in the study. Bottom: Prospective overview of the fate of drug, lipid, and surfactant during *in vitro* dispersion and digestion. Reproduced from Khan et al. (2018).

probability and rate at the lower grades. The best systems usually provide a transiently stable supersaturated drug solution that leads to enhanced oral absorption (Anby et al., 2012). Another approach is to maintain a supersaturated drug concentration in the LBF itself.

The LBF behavior in the gastrointestinal (GI) tract is extremely complex. The reason is that on one side, the LBF carries a significant diversity in its chemical composition: main lipid or lipid mixtures, co-solvents, surfactant mixtures, drug load. On the other hand, a time-dependent structural complexity is observed after dilution in the GI fluids. For example, the drug can reside in different colloid species (micelles, vesicles, emulsion droplets), it can precipitate in a crystal or amorphous state, it can form a meta-stable supersaturated solution, or a combination of the above (which is usually the case). A good illustration of the interplay between the excipients used for LBF formulation, solubility enhancement, precipitation and solid-state properties is provided in the recent publication by Khan et al. (Khan et al., 2018). The authors showed that the solid-state properties of a tolfenamic acid precipitate during an *in vitro* digestion assay of a LBF depend on its composition: an amorphous precipitate, linked to significantly higher solubility and supersaturation was obtained when a cationic surfactant was present in the LBF. Even more importantly, the mechanism of amorphization was related to the formation of an ionic liquid-type of associated between the oppositely charged drug and surfactant molecule, which demonstrates the multifaceted behavior of the system, see Fig. 3.

Therefore, an in-depth characterization of the behavior of LBF by *in vitro* dispersion and digestion methods, as well as by *in vivo* studies, in combination with the corresponding analytical techniques is required (Larsen et al., 2011; Jørgensen et al., 2018; Williams et al., 2012b). The latter have led to improved understanding of the system, which is used to guide the formulation optimization, as described in detail by Feeney et al. in a recent review (Feeney et al., 2016).

There are at least two drawbacks of LBF that need to consider in drug development. The first one is related to the problems with low drug loading in the LBF matrix, especially for brick dust molecules (*e.g.* itraconazole) that are characterized by low aqueous and lipid solubility. This challenge could be addressed by the hydrophobic ionic liquids concept, as shown by Williams et al. for itraconazole and danazol, which were dissolved in nicotinic acid-based ionic liquids and formulated as LBFs (Williams et al., 2014a). The other issue is related to the *in vitro-in vivo* correlation: as described in Section 5.2, the type of *in vitro* experiment and the media should be carefully selected in order to obtain good IVIVC. An industrial perspective on the current challenges in LBF development, such as oxidation stability of the drug, capsule compatibility, solidification of LBFs and others, is presented in the recent review by Holm (2019).

2.3. Avoidance of precipitation

Most of solubility enhancement techniques provide an increase in the apparent drug solubility by incorporation of drug molecules into nano-sized structures, such as mixed micelles, liposomes, and molecular inclusion complexes, or by using complex lipid vehicles (LBF). However, once the formulation enters the GI tract, it is diluted and encounters a complex environment of pH changes, hydrolytic enzymes and bile salts. The latter usually results in rapid loss of the solubilization capacity of the formulation and formation of a supersaturated metastable drug solution (Gao and Morozowich, 2006; Jannin, 2018). Supersaturated drug solutions can also be obtained when solid drug in ASD or cocrystal form is dispersed in the GI fluids (Frank et al., 2012a, b; Taylor and Zhang, 2016). The main characteristic of supersaturated solutions is the higher chemical potential of the drug molecules, compared to solutions at or below the equilibrium solubility. This feature can significantly increase the drug flux across the intestinal wall due to the higher concentration and the chemical potential gradient, thus increasing oral drug absorption (Anby et al., 2012; Gao and Morozowich, 2006; Taylor and Zhang, 2016). On the other hand, supersaturation can also cause drug precipitation (Mohsin et al., 2009) until the equilibrium solubility (determined with respect to the stable crystal form of the drug, see Section 4.1.) is reached. However, as long as the intestinal rate of absorption is higher than the rate of precipitation, drug absorption dominates, and *in vivo* precipitation will be limited. In addition, if the rate of precipitation is controlled and is sufficiently slow, the supersaturation window can significantly increase drug absorption and oral bioavailability. The concept of a drug formulation that provides significant and sustained supersaturation is termed “spring and parachute” approach (Brouwers et al., 2009). The approaches to prevent precipitation by using excipients, which provide the “parachute” effect, will be briefly described below. For more information about the nature of the supersaturated systems and their applications in drug delivery, the reader is referred to recent review articles (Brouwers et al., 2009; Laitinen et al., 2017; Taylor and Zhang, 2016).

There are two main approaches to avoid drug precipitation: (1) decrease the degree of supersaturation or (2) stabilize the supersaturated state by use of precipitation inhibitors. The first approach can be realized in several ways. For example, solubilizers which act as thermodynamically stable reservoirs for the drug molecules can be introduced (*e.g.* surfactant micelles, cyclodextrins) and some are natively present in the gut (bile salt aggregates, phospholipid vesicles) (Taylor and Zhang, 2016). Another way is to slow the drug release by using sustained-release formulations, so that a moderate supersaturation with lower drive for precipitation is maintained (Augustijns and Brewster, 2012). Such slow-release formulations may contribute an additional increase of oral bioavailability relative to their quick-release, non-precipitating counterparts (Six et al., 2005).

The most widely applied strategy to maintain supersaturation is to use precipitation inhibitors, such as polymers, low molecular weight surfactants (both as solubilization enhancer and precipitation inhibitor, Chen et al., 2015) or cyclodextrins (Brouwers et al., 2009). Among these, polymers are the most frequently used and the most studied ones (Warren et al., 2010). Several mechanisms of action have been proposed to explain the action of the precipitation inhibitors, all of which are related to modification of the nucleation and/or crystal growth stages by adsorption or complexation (Laitinen et al., 2017; Warren et al., 2010). Warren et al. studied a large set of polymers to unravel the link between the polymer molecular structure and its ability to sustain supersaturated drug solutions (Warren et al., 2013). Electrostatic attraction between the oppositely charged drug and the polymer was found to significantly delay precipitation, whereas the precipitation was induced when the species were similarly charged. Enhanced precipitation was observed also for polymers which are rich in primary amine, amide, carboxylic acid, and hydroxyl functional groups. Positive effect of ether groups was documented for halofantrine and meclofenamic acid.

Although the majority of studies in the area of polymeric precipitation inhibitors (PPI) are performed in the *in vitro* settings, a number of recently published investigations show that the PPI are also effective *in vivo* (Suys et al., 2018; Feng et al., 2018; Jaisamut et al., 2018; Quan et al., 2017). For example, Suys et al. determined the ability of a number of PPI to maintain the supersaturation during *in vitro* digestion of a fenofibrate LBF and then coupled the *in vitro* digestion assay with an *in situ* single pass rat intestinal perfusion model (Suys et al., 2018). The results showed good correlation between the PPI-mediated *in vitro* supersaturation and the *in vivo* exposure, thus confirming the functionality of the PPI and the relevance of the *in vitro* assay.

There are a number of drug delivery methods (ASD, LBF, some pharmaceutical salts) that yield supersaturated drug solutions after oral administration and have been described in Section 2.2. In addition, several excipient families that help to avoid drug precipitation in the gut are also available, the PPI being the most studied ones. In this respect, it is vital to keep in mind that the supersaturated state needs to be

stabilized only on a time scale relevant to drug absorption. Further *in vivo* and *in vitro* studies of supersaturated systems in non-sink conditions (Augustijns and Brewster, 2012; Sun et al., 2016) are required to precisely define the supersaturation window for easily permeable (BCS class II) and poorly permeable drugs (BCS class IV). Recently, a standardized method for assessing supersaturation propensity of drugs in biorelevant media has been developed. The method assesses the time for supersaturation (induction time) and the rate of precipitation at four degrees of supersaturation, the highest being the apparent maximum degree of supersaturation. By use of classical nucleation theory, the susceptibility of given drug to precipitation or supersaturation can be assessed, as well as the suitability for polymers to prolong the induction time or reduce the rate of precipitation (Palmelund et al., 2016; Plum et al., 2017).

2.4. Building of high absorptive concentration gradients

An important mechanism to promote absorptive flux of drugs is to achieve high drug concentration gradients across the intestinal wall (Brouwers et al., 2009). For poorly water-soluble drugs, there is a first formulation strategy that primarily aims at high luminal supersaturation of drug, while another approach emphasizes spatial aspects by targeting either specific segments of the intestine for site-specific absorption or the rationale is to target the mucosa specifically to achieve high local concentrations close to the brush border membrane of enterocytes. Regarding the first strategy of achieving generally high luminal drug concentrations, different supersaturating formulations are of interest, such as LBF, solid dispersions, or some colloidal delivery systems (Kawakami, 2012). Drug supersaturation is generally not only the result of formulation technology but is further determined by factors of the GI tract. There is a physiology-enabled supersaturation that is pH-driven and can be given with simple formulations of drug salts or it plays a role in supersaturating systems (Brouwers and Augustijns, 2014; Hens et al., 2016a; Kourentas et al., 2016; Brouwers et al., 2018). Such pH-driven physiology-enabled supersaturation can also occur with some comedication or following consumption of acidic beverages (Walravens et al., 2011; Knoebel and Larson, 2018).

Due to the metastable nature of drug supersaturation, the high concentrations should be sustained for sufficiently long to profit from high concentration gradients regarding absorptive flux. Therefore, it is the interplay of supersaturation, precipitation inhibition and absorption, which makes this formulation approach viable for biopharmaceutical challenging drugs. The solubilization and permeation effects have been modeled mathematically for cosolvent mixtures (Miller et al., 2012), micellar formulations (Miller et al., 2011), and there is further a model for the interplay of formulation digestion, supersaturation, and permeation (Stillhart et al., 2014). These models reveal some mechanistic complexity and they indicate, for example, that highest drug loading in the formulation may not generally entail optimal absorptive concentration gradients. Examples of *in vitro* experimental proof are found in Frank et al. (2012a) and Jacobsen et al. (2019).

To better understand how high absorptive concentration gradients are obtained, it is important to better know about intestinal formulation processing and hence the changes of any supersaturating system following oral administration. For lipid-based system, it is mostly the lipolysis-triggered changes that have to be considered and in case of solid dispersions, there should be sufficient knowledge about the different particles that evolve in the course of aqueous dispersion (Friesen et al., 2008). Following early experimental reports on the spontaneous formation of amorphous, drug-rich particles in aqueous dispersions of amorphous solid dispersions (ASDs; Tho et al., 2010), their impact on solubility (Frank et al., 2012b) and permeation (Frank et al., 2012a), the influence of drug-rich particles emerging from itraconazole solid dispersions was studied in more detail *in vitro* and regarding oral bioavailability in rats (Stewart et al., 2017a). The authors concluded that solid dispersions of BCS 2 drugs should be designed specifically for the emerging colloidal species so that high absorptive concentration gradients can be achieved. This is in line with research of Lynne Taylor's group in which effects of excipients and colloids were studied regarding membrane flux that is based on thermodynamic drug activity (Raina et al., 2015). Drug permeation through a membrane has been therefore used as a marker of thermodynamic drug activity that can be reduced not only by precipitation or a liquid-liquid phase separation but also because of strong drug-excipient interactions and slow partitioning from droplet or colloids, which may result during formulation processing in the GI tract.

A better understanding of emerging colloids from solid dispersions or LBF in the intestine is also important regarding local effects. It was, for example, shown that the acidic microclimate of the unstirred water layer can promote absorption from intestinal mixed micelles since fatty acids are better readily absorbed, which stimulates local supersaturation and high local concentration gradients for drug permeation (Yeap et al., 2013). Such local effects of colloids or particles from supersaturating formulations lead to the second strategy for high absorptive concentration gradients, in which spatial effects are targeted deliberately.

Drugs often exhibit regional differences of intestinal permeability so that site-specific concentration gradients are desirable to maximize oral bioavailability (Masaoka et al., 2006). An absorption window may be also widened as reported in the case of furosemide for which formulations of Eudragit L increased absorption in distal segments of the gastrointestinal tract (Terao et al., 2001). Site-specific concentration gradients have been targeted since many years to deliver anti-inflammatory drugs for inflammatory bowel diseases (Klein et al., 2005). Such controlled release systems, with targeting of an intestinal segment or colon, represent a comparatively established formulation approach (Basit and McConnell, 2011). By contrast, targeting specifically the mucus and the brush border membrane is a rather dynamic field of current research. The technical formulation challenges are here different for large active molecules as compared to small molecular drugs (Sigurdsson et al., 2013). Due to the importance of the topic, a separate section of this review is dedicated to mucus diffusion of drugs (5.5.). There is knowledge about molecular polymer properties required to achieve mucus adhesion (Peppas et al., 2009), which may be applied to the idea of supersaturating polymeric micelles (Yu et al., 2013). Such supersaturating colloids may emerge from LBFs or solid dispersions, which emphasizes again the idea that designed colloids from supersaturating formulations provide an attractive formulation rationale. In case of solid dispersions, any such system that combines amorphous solid dispersion with an intended controlled release behavior has been named previously a 4th generation of solid dispersions (Vo et al., 2013). More research in this field will be likely conducted in upcoming years with a particular aim to achieve high absorptive concentrations gradients.

3. *In silico* methods: computational modelling and simulation of performance

Computational assessment of drug performance in the GI tract has mainly revolved around drug dissolution/solubility and permeation, evaluations of disintegration and dissolution of, and release from, dosage form. Insights into solubility, permeability and combinations thereof have been extensively reviewed recently (Bergström and Larsson, 2018; Bergström et al., 2016; Matsson et al., 2016). Here we focus on presenting some of the recent work making use of molecular dynamics simulations (MDS) to understand intestinal solubilization and performance of advanced drug delivery systems such as LBF and amorphous solid dispersions (ASD).

MDS have during the last years gained interest in pharmaceutical sciences and entered into the analysis of dosage form performance. Molecular dynamic schemes can be implicit, *i.e.* the methods used are continuum-based and treat the surrounding solvent as an isotropic

continuous medium, or explicit, *i.e.* each solvent is presented at a molecular level. A drawback of the implicit simulation methods is that the atomistic level is lost. On the other hand, the explicit solvation methods, which explicitly consider solvent-specific effects and solute-solvent interactions and therefore, at least theoretically, be more accurate and provide better information about solvation, is computationally costly and simulations are time consuming. This is primarily because of the high degree of freedom from the explicit solvent molecules. More information around the MDS methodologies can be found in these recent papers (Bernardi et al., 2015; Szilárd et al., 2015; Ganesan et al., 2017). Performance evaluation in the GI tract is often simplified to evaluation of dosage forms in simple water models when studied computationally. However, relative solubilization of a number of drugs has been studied making use of MDS. Holmboe et al. made simulations of the lipid structures formed by bile components (phospholipids and taurocholate) and studied the partitioning of danazol, felodipine and carbamazepine into the lipid bilayers formed. It was concluded that the relative solubilization was strongly related to the capacity of the drug molecule to form hydrogen bonds with the taurocholate (Holmboe et al., 2016).

LBF is one advanced drug delivery system that has been studied by MDS with focus on dispersion and digestion of LBF. Pouton et al. have used MDS and all atom methodology to study phase changes upon dispersion and digestion and the resulting impact on solubilization. In one study they used danazol as the poorly water-soluble model compounds and solubilization capacity was simulated in response to digestion of a simple LBF (long-chain triglyceride) (Birru et al., 2017a). The simulations showed that the solubilization, and hence, the solubility, of danazol increased with increased digestion; these results were in agreement with the experimental data obtained for the same composition of digested material. In two other studies they explored how the digestion of lipids may influence the colloidal structures formed in the intestinal fluid, and to what extent cholesterol and pH influences the aggregation of intestinal lipids (Birru et al., 2017b; Suys et al., 2017). In a more recent study, the same group studied the location of probe molecules in a non-ionic surfactant (Warren et al., 2019). The micelles were composed of octaethylene glycol monododecyl ether, which has a C12 alkyl chain linked to a pegylated chain. The simulations showed that cyclic compounds were moved out from the micelle core, polar groups anchored the compounds in the micelle interface with the water and aromaticity resulted in exclusion of the compound from the micellar core. Drug localization has also been studied by Benson and Pless. They simulated mixtures of mono-, di- and triglycerides to mimic digestion and to what extent that influenced the location of the poorly water-soluble drug cyclosporine. It was shown that when the monoglyceride concentration increases the cyclosporine relocated to the core region of triglyceride moieties (Benson and Pleiss, 2014). A related study by Larsson et al. showed that the phase transitions that occur in response to dispersion of LBF in water can be reproduced using coarse-grained molecular dynamics (Larsson et al., 2017). Changing the resolution of the molecular structures from all atom to coarse grained results in that larger systems can be studied, and hence, *e.g.* solubilization of drug molecules can be studied under physiologically relevant conditions. In particular, relevant conditions of lipidic components (*i.e.* bile components and ingested lipids included in food or formulations) can be studied.

Another advanced formulation strategy that has been explored with MD simulations is ASD. Xiang and Anderson have studied such systems in a series of papers, with the main focus being on the physical stability of the ASD rather than the performance upon dissolution in *e.g.* the GI tract. They have studied different model compounds (indomethacin, ibuprofen, felodipine and the small peptide Phe-Asn-Gly) and polymers (hydroxypropyl methyl-cellulose, hydroxypropyl methyl-cellulose acetate succinate, poly(D,L-lactide), polyvinylpyrrolidone, polyvinylpyrrolidone-*co*-vinyl acetate, polyvinylalcohol) (Xiang and Anderson, 2004, 2005, 2013a, b, 2014, 2019) for properties such as internal hydrogen bond pattern, water mobility, glass transition temperature, mobility and miscibility. While some of the properties did not result in quantitative values in agreement with those determined experimentally (*e.g.* glass transition temperature) others were in good agreement with experimental data (*e.g.* water diffusion and identification of the functional groups (type and quantity) involved in the hydrogen bonds in the amorphous solid) (Xiang and Anderson, 2013a; Yuan et al., 2015). Performance-wise, Edueng et al. studied the mechanism of action for stabilization of ASD upon dissolution in water. They concluded that the hydrogen bond patterns between the drug-drug molecules, drug-water molecules and drug-polymer molecules were strongly influencing the extent to which a polymer will stabilize the supersaturation formed (Edueng et al., 2017). Similarly, Sun et al. used dissipative particle dynamics (DPD) to obtain molecular insights into the dissolution of lacidipine formulated with Eudragit E100 as an amorphous solid dispersion with 20% drug load. DPD is a coarse-grained strategy based on beads that clusters atoms and presents the molecular structure in a low resolution; these simulations can therefore be used to computationally study flexibility and mobility of long polymer chains over longer time spans. They found that the experimentally observed rapid release at pH 1.2 was a result of swollen microstructures whereas the slow dissolution at pH 6.8 was an effect of the formation of compacted microstructure of aggregated amorphous particles (Sun et al., 2017).

4. *In vitro* methods: compendial techniques used to evaluate the availability of small molecules from drug delivery systems

Techniques described in Pharmacopeia to evaluate the availability of poorly water-soluble drugs from drug delivery systems are limited to the measurement of drug solubility in various aqueous media and to the evaluation of dissolution/drug release from these systems.

4.1. Solubility measurement in buffers and biorelevant media

Solubility is the concentration limit, at thermodynamic equilibrium, to which a solute is uniformly mixed into a solvent (< 1236 > Solubility Measurements. 2017.Pharmacopoeia Forum. 43 (2), 1–17.). In common practice, thermodynamic solubility is also referred as equilibrium solubility (*i.e.* the concentration limit is reached at thermodynamic equilibrium) or saturated solubility (*i.e.* a saturated solution is used to ascertain that the concentration limit is achieved). Two other solubilities can be measured: apparent solubility and intrinsic solubility. Apparent solubility is the concentration experimentally measured of a solute in a solvent out of equilibrium conditions. The apparent solubility can be higher than equilibrium solubility if the drug delivery system generates supersaturation or it can be lower than equilibrium solubility if the time needed to reach equilibrium is insufficient. Intrinsic solubility is the concentration of the uncharged (neutral) solute in a solvent and can be measured in a specific pH range where the uncharged molecules are dominant.

In the current edition of the United States Pharmacopeia (USP) and European Pharmacopeia (EP) the method described to determine the solubility of drugs is limited to the evaluation of the 'approximate solubility' of the drug substance - the number of parts of solvent required to dissolve one part of solute (Description and relative solubility of USP and NF articles. 2018. United States Pharmacopeia 41 (First Supplement), 8516). In the table provided to describe solubility of drugs, the term 'poorly soluble' is not listed and other descriptive terms such as sparingly, slightly, very slightly soluble or practically insoluble are used.

Solubility can be performed in the dissolution medium described in the drug product monograph or in generic media such as simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) – the only two media purposely simulating gastrointestinal fluids currently listed in USP tests solutions (Test solutions. 2018. United States Pharmacopeia

Table 2
Compositions of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF).

Ingredient	Composition	
	SGF pH = 1.2	SIF pH = 6.8
Sodium chloride	2.0 g	–
Pepsin	3.2 g	–
Hydrochloric acid	7 mL	–
Monobasic potassium phosphate	–	6.8 g
Sodium hydroxide 0.2 N	–	77 mL
Pancreatin	–	10 g
Water	q.s. 1000 mL	q.s. 1000 mL

41, 5750–5761). Compositions of these two fluids are listed in Table 2. The main composition difference of these media with regular buffer solutions is the addition of enzymes (pepsin and pancreatin) that can modify the behavior of drug products, in particular those in gelatin capsules. However, these media cannot be considered as biorelevant as they do not contain any biliary components (phospholipids, bile salts, *etc.*) or food components.

A new monograph on solubility measurements is currently under revision (2017) for inclusion in USP. This new chapter describes factors affecting the solubility of drug in various aqueous media (*e.g.* pH, salts and counter-ions, co-solvents, surfactants) and the typical experimental methods used to assess drug solubility.

The USP monograph on solubility measurements recommends one method to measure equilibrium solubility of drugs, this is the saturation shake-flask method (2017). This method is reliable and widely used in the pharmaceutical industry to measure solubility of drug substance in aqueous media as well as in excipients (Williams et al., 2012b). The drug substance is added in excess to a solubility medium in a flask or vial. The suspension is mixed for 24 h in a temperature-controlled environment such as a shaking incubator. Then the excess (undissolved) solid is separated from the solution by sedimentation or centrifugation. The concentration of drug dissolved in the supernatant is assayed and equilibrium solubility is reached when multiple samples assayed after different equilibration time periods give equivalent results. The equilibrium solubility can be confirmed by assaying another sample after an additional 24 h of shaking, taking into account the stability of the medium. The same experimental set-up can be applied for the measurement of drug solubility in biorelevant media at 37 °C. The USP monograph on solubility measurements describes a series of methods to determine the apparent solubility of drugs, *e.g.* by potentiometric titration, turbidimetry. The monograph also addresses the miniaturization, high-throughput, and automation of these solubility measurements.

Biorelevant media have been developed to better estimate drug solubility in gastric or duodenal environment in the presence or absence of digested food components. Good correlation between solubilities measured in biorelevant media and in human gastrointestinal fluids has been reported (Vertzoni et al., 2005). To better match solubilities in human fluids various versions of biorelevant media were proposed and their physical-chemical properties (pH, surface tension, osmolality and buffer capacity) were adjusted (Dressman et al., 1998; Jantratid et al., 2008; Marques et al., 2011). Recently Markopoulos et al. (2015) proposed a decision tree to select the appropriate level of complexity of biorelevant media depending on the type of drugs, dosage forms and dosing conditions. Four levels of simulation of luminal compositions were proposed:

- Level 0 media are simple aqueous solutions where the pH is adjusted to mimic the pH of specific intestinal region. The compendial buffer solutions and SGF or SIF without enzymes described above could be used as level 0 media.
- Level I media mimic both the pH and buffer capacity of specific intestinal region.
- Level II media comprise in addition to above, bile components, dietary lipids, lipid digestion products and have an adjusted osmolality. These compositions better reflect the solubilization capacity of luminal fluids and the impact of fasted/fed dosing conditions.
- Level III media contain dietary proteins and enzymes (in place of digestion products from Level II) to address the impact of digestion and viscosity on the drug release.

The two most simple media (0 and I) are proposed for water-soluble compounds (BCS class I and III). Authors recommended the use of Level II media for the evaluation of the solubility of poorly water-soluble drugs (BCS class II and IV). Finally, the use of the most complex Level III media is proposed for enabling formulations where the composition can change overtime (*e.g.* LBFs) and to check the luminal stability of the drug and dosage form (Markopoulos et al. 2015).

Compositions of biorelevant media selected by USP (Jantratid and Dressman, 2009; Marques, 2011) are listed in Table 3.

It should be noted that the proposed USP compositions do not correspond to the biorelevant media recently reviewed by Markopoulos et al. (2015). The Level II compositions related to the USP proposed one

Table 3
Compositions of human biorelevant media. FaSSGF: Fasted-state simulated gastric fluid; FeSSGF: Fed-state simulated gastric fluid; FaSSIF-v2: Fasted-state simulated intestinal fluid (version 2); FeSSIF-v2: Fed-state simulated intestinal fluid (version 2); SCOF-2: Simulated colonic fluid (Jantratid and Dressman, 2009; Marques, 2011).

Ingredient	USP composition				
	FaSSGF pH = 1.6	FeSSGF pH = 5	FaSSIF-v2 pH = 6.5	FeSSIF-v2 pH = 5.8	SCOF-2 pH = 5.8
Hydrochloric acid	q.s. to pH 1.6	q.s. to pH 5	–	–	q.s. to pH 5.8
Sodium chloride	34.20 mM	237.00 mM	68.60 mM	125.50 mM	–
Sodium taurocholate	0.08 mM	–	3.00 mM	10.00 mM	–
Lecithin	0.02 mM	–	0.20 mM	2.00 mM	–
Pepsin	0.1 g	–	–	–	–
Sodium hydroxide	–	q.s. to pH 5	34.8 mM	81.65 mM	–
Sodium acetate	–	29.75 mM	–	–	–
Acetic acid	–	17.12 mM	–	–	170 mM
Milk, whole	–	1:1[a]	–	–	–
Maleic acid	–	–	19.12 mM	55.02 mM	–
Glyceryl monooleate	–	–	–	5.00 mM	–
Sodium oleate	–	–	–	0.80 mM	–

[a] Prepare the acetate buffer and mix 1:1 with whole milk.

Table 4
Selection of Level II biorelevant media compositions. FaSSGF: Fasted-state simulated gastric fluid; FeSSGF: Fed-state simulated gastric fluid; FaSSIF-v2: Fasted-state simulated intestinal fluid (version 2); FeSSIF-v2: Fed-state simulated intestinal fluid (version 2); FaSSCoF: Fasted-state simulated colonic fluid (Markopoulos et al. 2015).

Ingredient	Level II composition				
	FaSSGF pH = 1.6	FeSSGF middle pH = 5	FaSSIF-v2 pH = 6.5	FeSSIF-v2 pH = 5.8	FaSSCoF pH = 7.8
Hydrochloric acid	q.s. to pH 1.6	q.s. to pH 5	–	–	–
Sodium chloride	34.20 mM	181.70 mM	68.60 mM	125.50 mM	–
Sodium taurocholate	0.08 mM	–	3.00 mM	10.00 mM	0.15
Lecithin	0.02 mM	–	0.20 mM	2.00 mM	0.3
Pepsin	–	–	–	–	–
Sodium hydroxide	–	q.s. to pH 5	34.8 mM	102.40 mM	120 mM
Sodium acetate	–	32.98 mM	–	–	–
Acetic acid	–	18.31 mM	–	–	–
Lipofundin®/buffer	–	8.75/91.25	–	–	–
Maleic acid	–	–	19.10 mM	71.90 mM	75.8 mM
Glyceryl monooleate	–	–	–	5.00 mM	–
Sodium oleate	–	–	–	0.80 mM	0.1 mM
Tris					45.4 mM

are listed in Table 4. The main differences are highlighted by grey shading.

Recently another approach has been taken to simulate the composition of the gastrointestinal fluids. Based on literature reviews of the composition of intestinal fluids, design of experiments (DoE) have been applied to develop a set of media that can represent the environment that a drug is encountering during transit of the GI tract (Khadra et al., 2015; Madsen et al., 2017). This gives a reflection of the solubility of the drug in the intestinal environment and which factors (bile salt, phospholipids, pH, *etc.*) that are important for its solubility. Further, since DoE is used, an algorithm describing the solubility of the drug within the design space can be determined (Madsen et al., 2017).

4.2. Dissolution testing

During drug development, *in vitro* dissolution methods are applied to optimize the delivery profile of the dosage form. A prerequisite for the utility of this is that the selected *in vitro* methods are predictive of the *in vivo* outcome. The conventional dissolution equipments described in the current edition of the United States Pharmacopeia (USP) and European Pharmacopeia (EP) for the dissolution of drug substance from oral drug delivery systems are: basket (apparatus 1), paddle (apparatus 2), reciprocating cylinder (apparatus 3), and flow-through cell (apparatus 4) (< 711 > Dissolution. 2018. United States Pharmacopeia 41, 6459–6469). The choice of the apparatus and the dissolution media is specified in the individual monograph of dosage forms administered orally. Most commonly, dissolution tests are performed in either apparatus 1 or 2 with buffer solutions. These buffer solutions are selected to obtain sink conditions, *i.e.* conditions where the equilibrium solubility of the drug in the dissolution medium is at least three times higher than the actual drug concentration. These conditions are not possible with poorly water-soluble drugs when using simple buffer solutions (Phillips et al., 2012). Indeed, the volume of dissolution medium needed to dissolve the dose of the drug substance would be very high and more than the volume of the classic 1 L vessels. To overcome this limitation, various options are possible by selecting another apparatus or modifying the dissolution medium. The selection of apparatus 4 in the open loop configuration allows using large amount of dissolution medium and maintaining sink conditions in the flow-through cell where the dosage form is held. The modification of the dissolution medium can also increase the solubility of the drug substance by addition of surfactants (above their critical micellar concentration), complexing agent (*e.g.* cyclodextrins), or organic solvents (Phillips et al., 2012). The major drawback for these two options is that the dissolution medium composition and volume will not be biorelevant. Volumes of fluids in the gastrointestinal tract are relatively low (Schiller et al., 2005; Koziolek et al., 2014) in comparison to standard volume used in dissolution testing and do not contain organic solvent or complexing agents.

In the last decades, several new drug classification systems have been described to evaluate the potential difficulties of developing a given drug. These classification systems are the Developability Classification System (DCS) and the refined DCS, both based on the dissolution rate and solubility of the drug in biorelevant media, as well as the permeability (Butler and Dressman, 2010, Rosenberger et al., 2018). These classification systems can be considered as extensions/refinements of the BCS from 1995 (Amidon et al., 1995), which is only considering solubility in buffers. Applying the guidelines of these classification systems will facilitate identification of biopharmaceutics-related challenges using simple, well-defined methods and thereby evaluation of the likelihood of developing an *in vitro* biorelevant dissolution method, able to predict the *in vivo* performance (Rosenberger et al., 2018).

The selection of composition of biorelevant media (*e.g.* Level II biorelevant media, Georgaka et al., 2017), dissolution equipment and procedure to achieve *in vivo* predictivity, is not simple and should be based on the physicochemical properties of the drug and the desired delivery principle and therapeutic profile (Markopoulos et al., 2015; Madsen et al., 2017; Andreas et al., 2018; Löbenberg et al., 2000). Nevertheless, it is not always possible using conventional dissolution methods. Therefore, considering the many weak basic drugs in development in the pharmaceutical industry, dissolution models encompassing a gastric dissolution step and transfer to small intestinal conditions have been developed. These models often better predict the *in vivo* performance of weak basic drugs in immediate release formulations, as compared to conventional methods (Mathias et al., 2013; Kourentas et al., 2016c).

Biorelevant conditions are well adapted for some drug delivery systems where the drug is dispersed amorphous in a polymer matrix. The drug is then released from the matrix and generates a concentration of drug higher than its solubility in the dissolution medium (spring effect) and then tends to precipitate overtime to reach equilibrium solubility (parachute effect) (Brouwers et al., 2009). In these cases, the combination of biorelevant *in vitro* data with physiologically-based pharmacokinetic (PBPK) modelling is becoming more and more used, as it enables assessment of *in vivo* pharmacokinetics, based on *in vitro* drug behavior under physiologically relevant conditions (Kaur et al., 2018; Kostewicz et al., 2014). By basing PBPK models on biorelevant *in vitro* data from *e.g.* dissolution, solubility or supersaturation studies, it is possible to improve the ability to predict the *in vivo* performance

Table 5
Overview of gastrointestinal aspiration studies in healthy volunteers studying the intraluminal behavior of poorly water-soluble drugs (BCS Class II or IV).

Drug (API)	Dosage forms	Intake conditions[a]	Samples	Refs.
Amprenavir	Solution based on co-solvents and surfactant TPGS (1200 mg, Agenerase® capsule)	Fasted state	Duodenum, jejunum	(Brouwers et al., 2006)
	Prodrug (fosamprenavir 400 mg, Telzir® tablet)	Fasted *vs* fed state	Stomach, duodenum Systemic circulation	(Brouwers et al., 2007)
Itraconazole	Solid dispersion (100 mg, Sporanox® capsule)	Fasted state	Stomach, duodenum Systemic circulation	(Brouwers et al., 2017)
	Solution based on HP-β-CD (100 mg, Sporanox® solution)	Fasted state With *vs* without water intake	Stomach, duodenum Systemic circulation	(Berben et al., 2017)
Posaconazole	High-dose suspension (400 mg, Noxafil®)	Fasted state Water *vs* cola PPI comedication	Stomach, duodenum Systemic circulation	(Walravens et al., 2011)
	Low-dose suspensions (neutral *vs* acidified, 40 mg, Noxafil®), gastric dosing	Fasted state	Stomach, duodenum Systemic circulation	(Hens et al., 2016a)
	Low-dose solution (20 mg, Noxafil®), gastric dosing	Fasted state	Stomach, duodenum	(Hens et al., 2016a)
	Solid dispersion (100 mg, Noxafil® tablet)	Fasted *vs* fed state	Stomach, jejunum Systemic circulation	(Hens et al., 2016b)
Ketoconazole	Acidified solution (100 *vs* 300 mg), gastric dosing	Fasted state	Duodenum	(Psachoulias et al., 2011)
Dipyridamole	Acidified solution (30 *vs* 90 mg), gastric dosing	Fasted state	Duodenum	(Psachoulias et al., 2011)
Danazol	Solution in a liquid meal (150 mg), gastric dosing	Fed state	Duodenum	(Vertzoni et al., 2012)
Fenofibrate	Microparticles (200 mg, Lipanthyl® capsule) Nanoparticles (145 mg, Lipanthylnano® tablet)	Fasted *vs* fed state	Stomach, duodenum Systemic circulation	(Hens et al., 2015)
Abiraterone	Prodrug (abiraterone acetate 250 mg, Zytiga® tablet)	Fasted state Fed state	Duodenum Systemic circulation	(Geboers et al., 2016; Stappaerts et al., 2015)
Albendazole	Aqueous-based suspension (50 mg), with and without precipitation inhibitor Lipid-based suspensions	Fasted state	Stomach, duodenum	(Kourentas et al., 2016a)
Indinavir	Sulfate salt (400 mg, Crixivan®)	Fasted *vs* fed state PPI comedication	Stomach, duodenum	(Rubbens et al., 2016)
Diclofenac	Solution (50 mg, Cataflam®)	Fasted *vs* fed state PPI comedication	Stomach, duodenum	(van den Abeele et al., 2016)
	Potassium salt tablet (50 mg, Cataflam®)	Fasted *vs* fed state	Stomach, duodenum Systemic circulation	(van den Abeele et al., 2017b)
Ibuprofen	Tablet (800 mg)	Fasted *vs* fed state	Stomach, duodenum, jejunum Systemic circulation	(Koenigsknecht et al., 2017)

Abbreviations: PPI, proton pump inhibitor; HP-β-CD, hydroxypropyl-β-cyclodextrin; TPGS, d-α-tocopheryl polyethyleneglycol 1000 succinate.
[a] Unless otherwise stated, drugs were orally administered to fasted healthy volunteers with a standardized amount of water (180–330 mL). Fed state conditions resulted from intake of a liquid meal prior to drug administration.

(Berthelsen et al., 2014; Pathak et al., 2017). As an example, Hens et al. (2017b), applied *in vitro* data from biorelevant dissolution and supersaturation propensity studies, as well as clinical intra-gastric observations, to a PBPK model and achieved a prediction of the *in vivo* PK profile of posaconazole, dosed in two suspensions. Several PBPK models are available (Simcyp, GastroPlus), and are continuously being further developed (Kostewicz et al., 2014). However, more work is still needed in order to understand which *in vitro* data are needed and how to implement the data into the PBPK models.

However, these strategies cannot be yet applied to self-(nano) emulsifying drug delivery systems (S(N)EDDS) where the drug is already in solution and/or when the formulation can be degraded by enzymes (*e.g.* LBFs, SNEDDS). To address these limitations, advanced characterization techniques were developed such as solubility measurement/dissolution testing with human gastrointestinal fluids, lipolysis testing for evaluating the impact of lipases on LBFs, and combined dissolution/permeation techniques. These techniques will be described in the following chapters.

5. State of the art methods: evaluation of the absorption/availability of small molecules from drug delivery systems

5.1. Exploring the behavior of poorly water-soluble drugs in the human gastrointestinal tract

The intestinal absorption of poorly water-soluble drugs is challenging due to a limited intrinsic driving force for permeation across the intestinal mucosa. In most cases, absorption is also prone to substantial intra- and intersubject variability, since multiple gastrointestinal variables related to fluid composition, fluid volume, gastric emptying rate, and motility, may affect the behavior of poorly water-soluble drugs and the strategies that are used to enable their absorption. Therefore, an in-depth understanding of the interactions between drug, formulation and gastrointestinal physiology is critical to both the successful development of oral drug products and the efficient use of drug products in clinical practice. While multiple *in vitro* tools and *in silico* models are available to simulate gastrointestinal drug behavior, their biorelevance and predictive value often appear insufficient for poorly water-soluble drugs. Research approaches that enable the evaluation of drugs in the human gastrointestinal tract form the basis for an improved understanding of gastrointestinal processes critical for drug absorption and for a knowledge-based optimization of simulation tools.

While imaging techniques (*e.g.* scintigraphy, magnetic marker monitoring) are useful to monitor drug transfer and release, the evaluation of dissolution-related issues for poorly water-soluble drugs in the human gastrointestinal tract requires the aspiration and analysis of gastric and/or intestinal fluids following drug intake (Brouwers and Augustijns, 2014). Over the past decade, several gastrointestinal aspiration studies have been performed to study the intraluminal behavior of poorly water-soluble drugs (Table 5). Here, we will focus on a recent example that involves the gastrointestinal behavior of the highly lipophilic and weakly basic drug itraconazole after administration of either an HPMC-based solid dispersion (Sporanox® capsules) or a cyclodextrin-based solution (Sporanox® oral solution) to fasted healthy

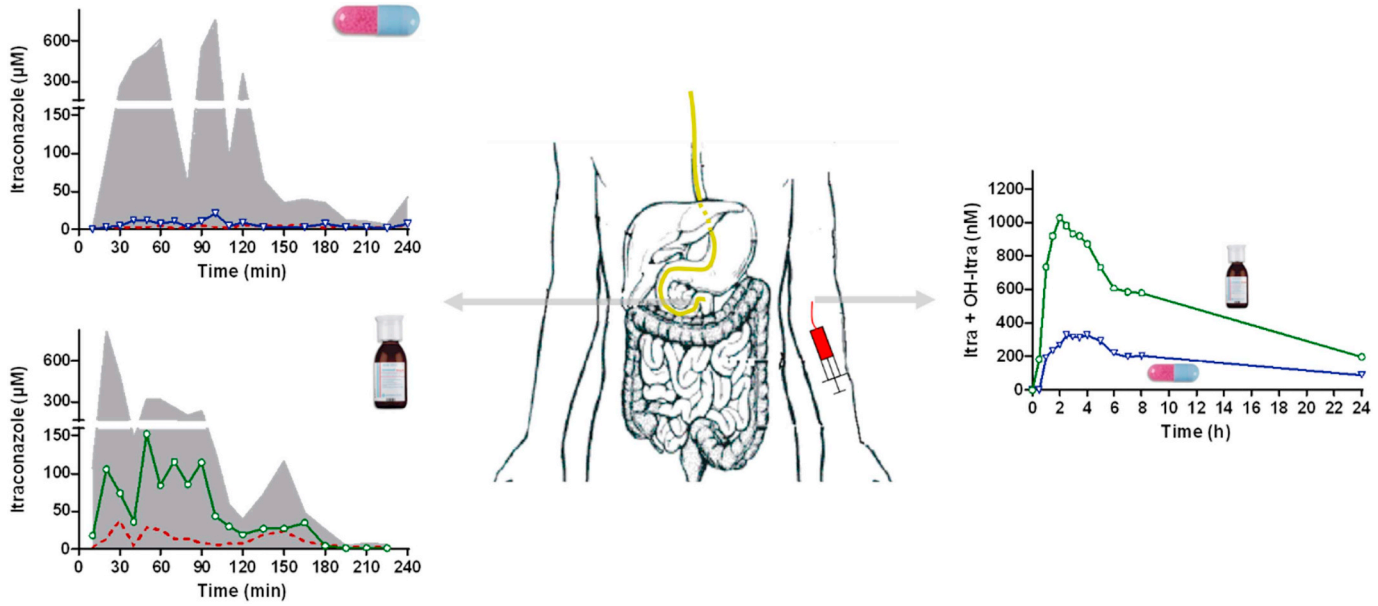

Fig. 4. Illustration of the gastrointestinal aspiration approach to study the intraluminal behavior and systemic absorption of itraconazole upon intake of a solid dispersion (Sporanox® capsules) or a cyclodextrin-based solution (Sporanox® solution). Left panel: mean concentration-time profiles of itraconazole in the human intestine (solid lines: itraconazole in solution, grey area: total itraconazole, red dotted line: itraconazole solubility). Right panel: mean concentration-time profiles of itraconazole in the systemic circulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.) Data from Brouwers et al. (2018).

volunteers (Fig. 4) (Brouwers et al., 2017). Using double-lumen tubes, positioned with the help of fluoroscopy, gastric and duodenal fluids were aspirated at predetermined time intervals and analyzed for (i) the total content of itraconazole (as solute and solid), (ii) itraconazole in solution, and (iii) the solubilizing capacity of the fluids for itraconazole. The obtained concentration-time profiles demonstrated that both formulations generated intraluminal itraconazole concentrations that substantially exceeded the solubilizing capacity of the fluids (*i.e.*, supersaturation), thereby enabling absorption. Compared to the solid dispersion, intake of the cyclodextrin-based solution resulted in a much higher dissolved fraction of itraconazole in the duodenum, explaining the improved absorption seen in the simultaneously assessed systemic concentration-time profiles (Fig. 4).

The gastrointestinal concentration-time profiles of poorly water-soluble drugs in humans may act as excellent reference data to optimize *in vitro* and *in silico* simulation tools. For instance, data on the intestinal precipitation of posaconazole in humans have been used to validate a physiologically based pharmacokinetic model for drug dissolution, supersaturation and precipitation (Hens et al., 2017b). Also, the value of the Biorelevant Gastrointestinal Transfer (BioGIT) system to predict the dissolved fraction of posaconazole and itraconazole in the upper small intestine has been confirmed by comparison with intestinal concentration-time profiles obtained in humans (Kourentas et al., 2016b).

The simultaneous assessment of gastrointestinal and systemic concentrations further helps to identify the factors and processes critical for absorption. For instance, intestinal concentrations of itraconazole after intake of the above-mentioned cyclodextrin-based solution were substantially reduced by the intake of additional water (Berben et al., 2017). However, the systemic exposure to itraconazole was not affected by water intake. Without water intake, the cyclodextrins present in the formulation were less diluted and stronger higher-order inclusion complexes were formed with itraconazole in the small intestine, thereby reducing precipitation and increasing concentrations. However, these stronger complexes also reduced the apparent permeability of the intestinal mucosa for itraconazole (entrapment), as was demonstrated by determining the permeation of itraconazole from the aspirated fluids across Caco-2 monolayers. This illustration of the interplay between solubility and permeability in the human gastrointestinal tract stresses the need to implement permeation testing into formulation evaluation when working with solubilizing approaches. As such, these observations stimulated the further development of cell-free permeation tools than can be easily implemented into traditional dissolution testing (Berben et al., 2018a; Berben et al., 2018b).

Overall, the profiling of drug concentrations in the upper gastrointestinal tract provides a unique insight into the processes underlying intestinal drug absorption. The added value of this approach for drug development primarily lies in guiding the physiologically-based optimization of *in vitro* and *in silico* simulation tools. To this end, it is critical to link the observed intraluminal drug behavior to important gastrointestinal variables. Using the aspiration approach, these variables are limited to the composition of the fluids (pH, bile salts, phospholipids, food (digestion) products). Therefore, advanced approaches are being explored to monitor additional gastrointestinal variables in parallel. In this respect, recent advances in combining fluid aspiration with manometry to monitor gastrointestinal motility (Bermejo et al., 2018; van den Abeele et al., 2017a) offer new opportunities to fully elucidate the interaction between drug products and the gastrointestinal environment.

For obvious ethical reasons, gastrointestinal aspiration studies can only be performed in healthy adults, and not in specific patient populations including pediatric and geriatric patients. Making the *in vitro* and *in silico* simulation of gastrointestinal drug behavior also relevant for special populations is currently a major challenge. To this end, it is essential to use patient data to characterize the gastrointestinal physiology in these populations, as has been done recently with respect to gastric fluid composition in neonates, infants and children (van den Abeele et al., 2018). Based on such data, the sensitivity of the drug product behavior to relevant population-dependent deviations in gastrointestinal physiology can then be evaluated in simulation tools.

5.2. *In vitro digestion evaluation*

Ingestion of lipids induces a two-way interaction between the formulation and the GI tract. On one hand, lipids are digested by various

lipases secreted by the stomach and the pancreas to transform lipophilic compounds into more hydrophilic ones (N'Goma et al., 2012). The modification of the chemical composition of the lipids – even lipid-based surfactants that are esters of synthetic polymers such as polyethylene-glycols can be digested (Fernandez et al., 2007; Fernandez et al., 2008) - also affects their colloidal structures and favors the formation of mixed vesicles, lamellar phases and micelles (Chamieh et al., 2017; Fernandez et al., 2013; Müllertz et al., 2015; Vithani et al., 2017; Vithani et al., 2019). On the other hand, the presence of lipids will modify the gastrointestinal physiology by slowing down the gastric emptying, stimulating the synthesis and secretion of bile, and the secretion of pancreatic juice. All these events can affect the intraluminal performance of LBFs and their ability to solubilize the drugs in colloidal structures (Feeney et al., 2016).

To better anticipate the effect of lipolysis on the performance of lipid containing drug delivery systems, a digestion test has been proposed using a pH-stat apparatus. This device has been used by the food and biochemical fields for decades and was transposed to the pharmaceutical field in the last two decades. Lipids or LBF can be dispersed in a lipolysis medium mimicking the gastric or intraluminal content with lipases to start the lipolysis of ester-based compounds. The release of fatty acids by lipases can decrease the pH of the medium depending on their pKa and the pH-stat apparatus allows maintaining the pH constant by addition of sodium hydroxide with an automated burette (Bakala-N'Goma et al., 2015). Various lipolysis media compositions and operating conditions have been described in the literature to either mimic the fasted (Sassene et al., 2010) or fed (Fernandez et al., 2009) state human GI conditions. An international LFCS consortium proposed a standardized and validated test using the pH-stat apparatus to study the lipolysis of LBFs in the small intestine (Williams et al., 2012b). They described the composition of the lipolysis medium to mimic the fasted duodenal conditions in term of calcium and pancreatin (lipases) concentration (Sassene et al., 2014), as well as the type and concentration of bile salts (Williams et al., 2012a). Another harmonized test has been proposed by the COST action INFOGEST for the food industry with a three steps lipolysis protocol – oral (mouth), gastric and intestinal phase (Minekus et al., 2014). This static *in vitro* simulation of gastrointestinal food digestion has been recently amended and improved by the inclusion of gastric lipase during the gastric step (Brodkorb et al., 2019). Indeed, this improvement has been made possible by the recent commercial availability of gastric lipase in rabbit gastric extract (since 2018) and the validation of the utility of this extract to mimic the human gastric digestion phase of both lipids and proteins (Capolino et al., 2011; Sams et al., 2018). An update of the LFCS protocol for the evaluation of LBFs has not yet been proposed. However, the behavior of LBFs in fed conditions is highly relevant as the increased pH in the stomach will favor the action of the gastric lipase and preduodenal digestion of the formulation with potential precipitation of the drug. This would lead to a decreased amount of solubilized drug reaching the small intestine and could preclude its absorption.

The lipolysis test is mainly currently used to evaluate the ability of the LBF to transfer the drug in solution from the dispersed LBF toward the formed colloidal phases and finally micellar solubilization (Feeney et al., 2016; Mu et al., 2013). The drug can either be dissolved in micelles, in supersaturation in the aqueous phase or precipitate in its crystalline or amorphous form. Various techniques have been used to follow the evolution of the colloidal phases – cryogenic transmission electron microscopy (cryo-TEM) (Tran et al., 2017b), *in situ* synchrotron small-angle X-ray scattering (SAXS) (Tran et al., 2017b; Vithani et al., 2017), dynamic light scattering (DLS) or Taylor dispersion analysis (TDA) (Chamieh et al., 2017) - and the fate of the drug - chromatographic assay (Jannin et al., 2015), Raman spectroscopy (Stillhart et al., 2013), and cross polarized optical microscopy (Williams et al., 2013). Depending on the amount of drug in colloidal solution after 1 h of lipolysis, the performance of the formulation can be classified toward its precipitation propensity (Williams et al., 2014b). It is assumed that the drug solubilized in the colloidal phases will be more readily available for absorption and thereby favor *in vivo* drug exposure, while the precipitated drug will not be able to dissolve in the luminal content and will not be available for absorption. This is not the case for some drugs that are precipitating in the amorphous form from long chain LBFs like cinnarizine (Sassene et al., 2015) and thus easily re-dissolve in the lipolysis medium (Sassene et al., 2010).

Following that consideration, some *in vitro-in vivo* correlation studies have been conducted and led to ambiguous results with good rank order correlation for danazol (Cuiné et al., 2007) or griseofulvin (Dahan and Hoffman, 2007), and no correlation for fenofibrate (Griffin et al., 2014; Tran et al., 2017a). One explanation of the lack of correlation is the missing absorption step in the existing *in vitro* lipolysis models, this is especially important for readily absorbed drugs, for which the digestion of the excipients, and subsequent drug solubilization in the digested media, are of minor importance (Griffin et al., 2014; Larsen et al., 2013). Another issue is the species-specific consideration, *e.g.* application of an *in vitro* lipolysis model that simulate human intestinal conditions to compare with a pharmaco-kinetic study carried out in a preclinical species, *e.g.* the rats (Anby et al., 2014, Siqueira et al., 2017). Based on these observations, Jørgensen et al. (2018) developed an *in vitro* lipolysis model, simulating the rat GI tract. The model also included a gastric emptying step and was shown to better correlate with the *in vivo* pharmacokinetic profile. The simulation of the performance of LBF, could also be significantly improved if the data could be incorporated into PBPK models, however, this, however, is not yet possible.

To conclude this section, the current two main limitations of the *in vitro* lipolysis test are the absence of gastric digestion phase and the lack of drug absorption from the lipolysis vessel to better anticipate drug partitioning. The first limitation should be solved in the near future as gastric lipase is now commercially available (*e.g.* rabbit gastric extract). Solutions to the latter limitation will be discussed in the following chapter as promising results were obtained by Bergström and coworkers very recently (Keemink and Bergström, 2018; Keemink et al., 2019).

5.3. Coupling techniques: dissolution/permeation

Common *in vitro* approaches to predict the biopharmaceutical performance of enabling formulations measure drug solubility- or dissolution rate - improvement with regard to “apparent” solubility. For a more detailed discussion of the terms apparent and molecular solubility see Section 5.4. There is growing evidence, however, that the rate and extent of drug absorption does not always correlate well with the concentration of apparently dissolved drug. Since oral absorption is a process in which dissolution and permeation occur concurrently and sequentially across intestinal barrier, *in vitro* models that combine dissolution/permeation (D/P) are considered promising to mimic the *in vivo* absorption process. The D/P approach was first introduced by Ginski and Polli (Ginski, 1999), and since then many variants have been developed at different levels of complexity to meet specific objectives, such as estimating the effect of pH change (Liu et al., 2013), food intake (Kataoka et al., 2011), and the dosage form (Motz et al., 2007) on the oral absorption of drugs. While common permeation screens (Caco-2, PAMPA, PVPA) in general demonstrated good *in vitro-in vivo* correlations when testing pure drug compounds, up to now there is only limited experience with the application of D/P-models for enabling formulations (Kataoka et al., 2006). Several obstacles seem to have hampered a faster development. The standard cell-based permeation models, such as *e.g.* Caco-2 cell monolayers, have some limitations for formulation screening. They typically cannot resist the solubilizing additives in *e.g.* LBFs (Bibi et al., 2015), however, a recently developed dynamic digestion-permeation assay making use of immobilized enzymes for digestion has proven compatible with a range of such formulations (Keemink and Bergström, 2018; Keemink et al., 2019). Other drawbacks are that they require long cell incubation times (21 days),

Table 6
Overview of combined dissolution/permeation testing set-ups.

Setup	Type of barrier	A [cm^2]	V_{Donor} [mL]	A/V [cm^{-1}]	Sample	Type of coupling of dissolution with permeation	Reference
IDAS2	Caco-2 cell monolayer	2.26 (2 × 1.13)	500	0.005	Complete dosage form	Direct	Li et al., 2019
MacroFLUX™	hydrophobic polyvinyliden-fluoride membrane-impregnated with lipid	3.80	1062	0.004	Complete dosage form	Direct	Borbás et al., 2018
Microflux™	hydrophobic polyvinyliden-fluoride membrane-impregnated with lipid	1.54	20	0.08	Drug substance or downscaled formulation	Direct	Tsinman et al., 2018
Side-by-side	Biomimetic Permeapad® barrier	1.77	5.00	0.35	Drug substance or downscaled formulation	Direct	Jacobsen et al., 2019
Vertical membrane flux cell in µDiss profiler	Membrane with lipid-filled pores (PAMPA?)	4.90	5	0.98	Drug substance	Direct	Stewart et al., 2017b
Coupled dissolution – mass transfer setup	Dialysis membrane: cellulosic hollow fiber module	100	50	2.00	Supersaturated solution	Direct	Hate et al., 2019
PermeaLoop®	Biomimetic Permeapad® barrier or dialysis membrane	27.6	20	1.38	Downscaled dosage form	Direct	Sironi et al., 2018
Permeapad® plain plate	dialysis membrane	0.2	0.15–0.4	1.33–0.5	Drug substance or downscaled formulation	Direct	Jacobsen et al., 2018
AMI-system	Dialysis membrane	4.91	0.7	7.38	Complete dosage form	No direct coupling	Berben et al., 2018a, b

Where V is the typical volume of the donor compartment, A the effective permeation area and A/V the area-to-volume-ratio.

and that the poor inter-laboratory comparability make it difficult to compare results produced in different laboratories (Hayeshi et al., 2008). Non-cellular barriers, such as parallel artificial membrane permeability assay (PAMPA) and phospholipid vesicle-based permeation assay (PVPA) (Flaten et al., 2006b), are promising alternatives for assessing passive, transcellular permeability and have recently been employed in D/P setups (Berben et al., 2018a; Gantzsch et al., 2014; Kataoka et al., 2014). Although contradicting and alternative findings have been reported, PAMPA and PVPA appear to have the inherent risk of lacking functional stability against numerous solubilizing excipients typically used in enabling formulations as well as biomimetic media. Furthermore, PAMPA lacks biomembrane-like structures, making it less bio-mimetic and for PVPA a laborious preparation procedure needs to be adopted to overcome limited functional stability and short shelf-life (Flaten et al., 2006a).

In 2016, Di Cagno and Bauer-Brandl proposed a new biomimetic barrier, Permeapad® (Di Cagno and Bauer-Brandl, 2016), for a fast and reliable determination of drug passive permeation properties (Di Cagno et al., 2015). Recent pilot studies, employing the Permeapad® within the commonly used side-by-side diffusion chamber, indicate a great potential to specifically assess enabling formulations D/P-performance, since the barrier is compatible with a wide range of surfactants, co-solvents, and biomimetic media and even withstands lipolysis conditions used for SEDDS and SNEDDS (Bibi et al., 2017; Flaten et al., 2006a). At the same time, these studies revealed a limitation of the conventional side-by-side diffusion chamber geometry in terms of permeation surface area. It has been postulated that a significantly different design, with a larger barrier interface, is needed, assuming that a larger barrier interface to volume ratio will enable to study the mutual interplay of dissolution rate with corresponding barrier flux under non-steady state conditions, where dissolution is rate limiting as it is the case *in vivo* (Sironi et al., 2017a; Sironi et al., 2018). During the past two years, several groups have suggested a range of alternative experimental set-ups for combined dissolution/permeation testing; these are summarized in the table with regard to the type of barrier used and setup-geometry, *i.e.* donor volume, effective barrier area, and area/volume-ratio (Table 6).

Depending on geometry and permeability of the drug compound across the chosen barrier, different scenarios of interplay between absorptive flux (or mass transfer) and dissolution (or release) can be expected: For very low A/V-ratios (0.1 and below) the fraction of drug removed from the dissolution chamber during the entire duration of the D/P-experiment typically is negligible. Still, such approaches are deemed useful because they may report the “fraction of permeable drug”, which often is different from the fraction of (apparently) dissolved drug. For high A/V-ratios (1 and above), a significant feed-back of absorptive flux (mass transfer) on drug dissolution and/or supersaturation has been documented (Sironi et al., 2017a; Hate et al., 2019), although the fraction of absorbed (transferred) drug still mostly is low in comparison to that observed *in vivo*. A substantially higher supersaturation has been observed in the presence of an absorptive compartment (than in the absence) for a fenofibrate nanoformulation (Sironi et al., 2017b), despite the fact that the A/V-ratio in the side-by-side setup employed was moderate (0.35).

5.4. Differentiation between apparently dissolved drug and molecularly dissolved drug

In order to rank enabling formulations by biopharmaceutical performance prediction from *in vitro* experiments, typically drug solubility improvement is employed.

It is useful to differentiate between “molecularly” dissolved and “apparently” dissolved drug molecules. “Molecularly” dissolved solutes are “free”, *i.e.* individual molecules surrounded by their hydration shells (Frank et al., 2012a). The term “apparently dissolved” includes - in addition to the molecularly dissolved fraction - the solubilized

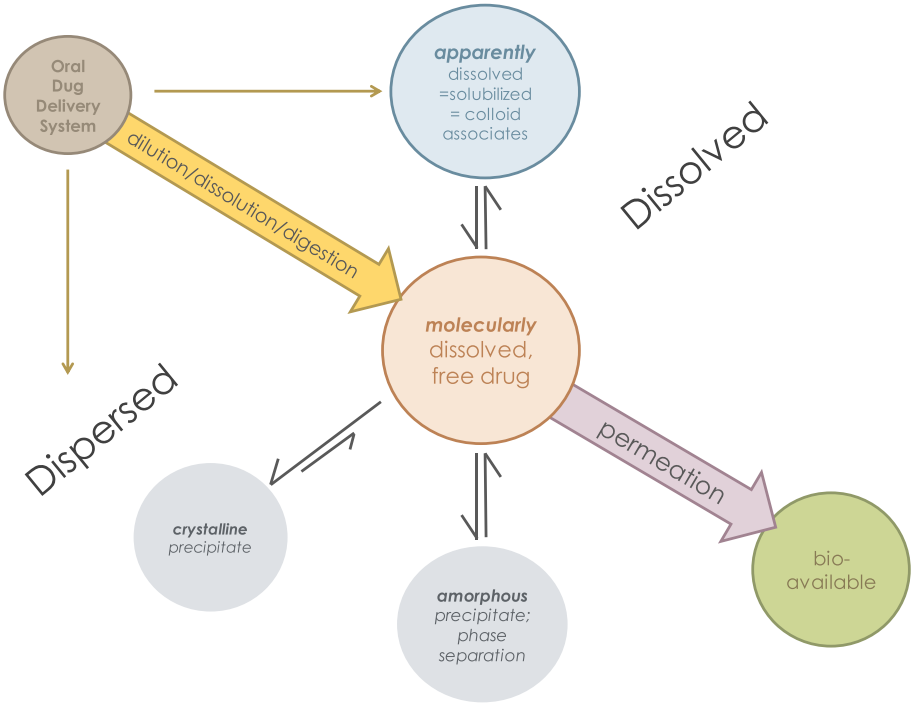

Fig. 5. Schematic representation of the impact of molecularly dissolved drug fraction and other states on bioavailability of oral drug delivery systems.

fraction of drug molecules that are present in various colloidally associated or complexed states (Augustijns et al., 2014; Buckley et al., 2013). Relevant colloids in this context are micelles, mixed micelles, and vesicles formed by formulation additives or those present in human or artificial (biomimetic) dissolution media (Elvang et al., 2016; Elvang et al., 2018). Free drug molecules and those associated with colloids behave differently in both thermodynamic (solubility) and kinetic (dissolution rate; permeation) settings. This is of particular significance with respect to bioavailability of enabling formulations permeation (absorption). It has been hypothesized that the "molecularly" dissolved drug molecules, but not the colloidal drug associates, permeate across biological barriers; the "apparently" dissolved drug fraction appears not to correlate well with permeability (Buckley et al., 2013; Fong et al., 2017a). This means in other words that absorption/permeation rates would essentially depend on the molecularly dissolved drug concentration. During the time course of a dissolution experiment for oral drug delivery systems, the free drug concentration depends on molecular solubility, and the kinetics of distribution of molecules in and out of the respective colloidal adducts and other particles (Fig. 5).

Molecular drug concentration would thus probably be a better predictor of absorption and bioavailability than the apparently dissolved concentrations and apparent solubility (Frank et al., 2012a). However, in literature, with few exceptions, apparently dissolved drug concentrations are reported because they are experimentally readily accessible by filtration or centrifugation, whereas assay of the molecularly dissolved drug concentration is done by dialysis methods, where it takes several hours/days to reach equilibrium.

However, *in vivo* oral drug delivery systems meet rapid dynamic changes of environment during their GI transit - the time period of which in total is 2–6 h, including pH jump, mechanical exposure, food effects, digestion fluids *etc.* While the conditions in the GI fluids continuously change, drug molecules are exchanged between molecularly dissolved, colloid-associated and particulate states. Simultaneously, free drug molecules are absorbed and thus eliminated from the media. Therefore, in many cases, when starting from a solid formulation, solubility limits for the API (apparent solubility) are not reached *in vivo*, making (apparent) solubility a weak tool to predict biological performance.

An approach to circumvent the outlined difficulties is to develop *in vitro* models mimicking the interdependent dynamics of dissolution and elimination/permeation processes (see Section 5.3 on combined dissolution and permeation) possibly allow to follow supersaturation and change in solubilization through media composition changes and simultaneously decreasing the free drug concentration by a permeation (absorption) sink. In such experiment, changing compositions, rates of transport *etc.*, need to be in a realistic balance to mimic the dynamic interrelationship between colloids, molecules, precipitates, and permeates. If the chosen conditions are suitable, it is a pragmatic approach for screening and ranking of formulations to look at permeation rates and quantities through biomimetic barriers, because such set-up already implies the distinction and dynamic interrelationships between molecular and apparent drug concentrations.

If, however, a better mechanistic understanding is desirable to make rational decisions and to optimize formulations, on-line time-resolved measurement of molecularly dissolved drug fraction evolving from a drug delivery system in a dynamic model is needed. Recently, it has been suggested to employ microdialysis to address this challenge (Fong et al., 2017b). While microdialysis has already been established for *in vivo* (animal) studies and clinical settings to measure drug exposure in tissues, its use for *in vitro* dissolution and dissolution-/permeation methods is in its infancy. Koplin et al. have refined the suggested approach and demonstrated the implication of colloidal solubilization on *in vitro* extraction rates (Koplin et al., 2017).

There is currently very limited experience with D/P-systems comprising on-line assay of the molecularly dissolved drug concentrations. First, D/P-systems in conjunction with microdialysis-sampling should enter broader use and more data should become available from *in vitro* screening of enabling formulations with established *in vivo* performance. Then it may become possible to conclude if this approach helps to find the optimum formulations faster and more reliably thereby tremendously reducing the number of animal studies.

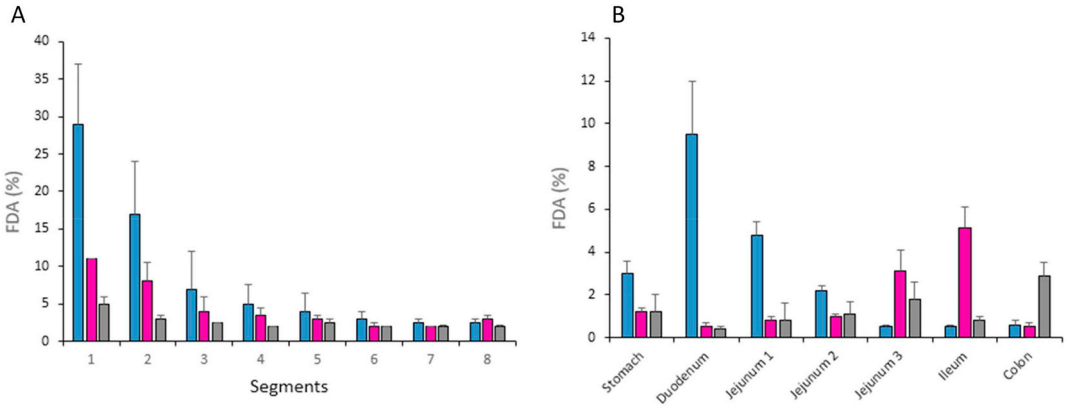

Fig. 6. A: Penetration capacity of fluorescein diacetate (FDA) labeled papain decorated microparticles (blue bars), bromelain decorated microparticles (red bars) and undecorated microparticles (grey bars) analyzed *via* rotating silicon tube method at 37 °C for 4 h; B: Percentage of FDA incorporated in papain decorated microparticles (blue bars), bromelain decorated microparticles (red bars) and undecorated microparticles (grey bars) remaining on rat gastrointestinal mucosa within 3 h after oral administration; adopted Mahmood et al., 2017. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

5.5. Mucus diffusion

The mucus gel layer covering the GI epithelium represents a further barrier for orally administered therapeutics and in particular for micro- and nanocarrier systems that are supposed to overcome it. Generally, the mucus barrier restricts drugs and drug delivery systems on their way to the absorption membrane by its adhesive and viscous properties. The adhesive properties of mucus are, on the one hand, related to (I) its anionic character due to sialic- and sulfonic acid substructures ionically binding cationic drugs and drug carriers (Crowther and Marriott, 1984), (II) its hydrogen bonding properties due to oligosaccharide substructures (Braybrooks et al., 1975) and (III) its partially lipophilic character due to fatty acid substructures being responsible for hydrophobic interactions (Lichtenberger, 1995). The viscous properties, on the other hand, are mainly based on mucus glycoproteins exhibiting cysteine-rich subdomains that are connected with each other *via* disulphide bonds building up a stable three-dimensional network structure with a mesh size in the range of 10–200 nm. The larger the diameter of drugs or carrier systems, the more is their diffusion across the mucus gel layer hindered (Desai et al., 1992; Griesser et al., 2018). To which extent the adhesive and to which extent the viscous properties of the mucus barrier are responsible for the reduction in the diffusion rate, however, is difficult to determine.

Methods that are useful to evaluate the mucus permeation behavior of drugs and drug delivery systems *in vitro* include over all equilibrium dialysis, rheological studies and mucus diffusion studies. Crucial for all these techniques is the use of freshly isolated native mucus. Methods to isolate mucus form porcine and bovine GI-tract have been described in detail previously (Allen et al., 1989).

To evaluate the binding and interactions of drugs and carrier systems with mucus equilibrium dialysis and rheological studies are recommended. Most commonly, isolated mucus is loaded into dialysis sacs and immersed in a buffer system containing the drug of interest. Once equilibrium has been reached the extent of binding to the mucus can be determined (Crowther and Marriott, 1984; Kearney and Marriott, 1987). Alternatively, rheological synergism has been proposed as an *in vitro* parameter to evaluate drug or drug delivery system – mucus interactions: the higher the rheological synergism, the stronger are these interactions (Marriott and Kellaway, 1975; Müller et al., 2013).

In contrast to mucus equilibrium dialysis and rheological studies, diffusion studies are more informative. In its simplest form, a cylindrical diffusion chamber is filled with mucus and on one end the drug or nanocarrier system of interest is applied. After incubation of at least several hours up to several days at 37 °C, the amount of drug or nanocarrier is quantified in pre-determined segments of the cylinder (Larhed et al., 1997; Pereira de Sousa et al., 2015). Although the method is comparatively simple, it has the disadvantage that at short incubation times of just a few hours it is difficult to discriminate the diffusion behavior of different nanocarriers. On contrary, long incubation times of several days are time consuming and a decrease in mucus stability over time has to be taken into consideration. In Fig. 6 an example of results obtained with this method is provided. Particles having been decorated with different mucolytic proteases showed in this test a mucus permeation behavior that correlated very well with *in vivo* results (Mahmood et al., 2017).

In contrast to permeation studies utilizing mucus sandwiched between filters facing the donor and acceptor compartments (Desai et al., 1992; Friedl et al., 2013), the method is independent from parameters that are difficult to control such as the thickness of the mucus and unstirred water layer or membrane effects of the filter. Furthermore, *via* this method even the self-diffusion coefficient of the diffusing species in mucus can be determined rather than just the percentage of drug or carrier system having permeated the mucus layer in a certain time period in dependence on the applied concentration gradient. Furthermore, even the impact of water movement through the mucus, as it is the case in the intestine because of extensive water absorption, can be simulated (Fabiano et al., 2017). Microscopic techniques are also based on mucus diffusion studies allowing a more rapid determination of the diffusion coefficient (Henry et al., 1992). *Via* video microscopy followed by post-acquisition analyses the diffusion coefficient even of nanocarriers in mucus can be determined. As several particles can be simultaneously tracked *via* this technique, it is also known as multiple-particle-tracking (MPT) (Crocker and Grier, 1996). In order to visualize nanocarriers in the mucus they have to be labeled on their surface preferably by fluorescence dyes. Apart from microscopic techniques the diffusion of drugs and carrier systems in mucus can also be analyzed *via* pulsed-gradient spin-echo NMR (PGSE-NMR) (Occhipinti and Griffiths, 2008). In contrast to microscopic techniques a fluorescence labelling of the drug or of the carrier system, with all its drawbacks such as altering the original properties of diffusing species because of the labelling or an unintended release of the label causing misleading results, is not needed. The likely only A drawback is that the sample must contain a spin-active nucleus such as 1H or 19F. Furthermore, the method is comparatively more complex than all other techniques depending on high-tech equipment.

5.6. In vitro and ex vivo absorption evaluation: cell-based models and Ussing chambers

Despite the presence of numerous *in vitro* models for evaluating PWSDs behavior (see compendial and state-of-the-art methods described in previous chapters), *in vitro* cell-based and *ex vivo* methods provide a compromise between the complex and expensive *in vivo* models and the simplicity and one-dimensional feature of *in vitro* models. *In vitro* cell based models provide an intermediate complexity and resemble the *in vivo* system more closely. Furthermore, they also allow studying the interaction of these systems with human-originated cells and tissues that include the transport systems in the human intestine. Furthermore, simple buffers can also be replaced with simulated intestinal fluids for adding another component to the system (Antoine et al., 2015).

Starting with the cell-based systems, the ability of Caco-2 based systems to form polarized monolayer with transporters, tight junctions, mucus layer (with HT29-MTX cells) and M-cells (with Raji B cells) provide a wide array of models with which the advanced drug delivery systems can be evaluated. In addition, these models can also be used to evaluate the transport mechanism by using specific inhibitors. Also, for *ex vivo* models, the possibility to use human intestinal tissue provides more information on how the system would behave in the *in vivo* conditions. Finally, the Ussing chambers system allows using different segments of GI tract, giving information on how the drug and the delivery system interact with these specific segments.

Both systems, especially cell-based models, have been used extensively to evaluate different advanced drug delivery systems such as polymeric nanoparticles, lipid nanoparticles, silica and silicon nanoparticles, nanocomplexes, *etc.* The ease of establishing the system and performing the experiments allow us to easily evaluate advanced drug delivery systems. Some examples of information that can be gathered from these systems include: (i) absorption behavior and permeability, (ii) interaction with the mucus layer, (iii) interaction with the cell monolayer, (iv) mechanism of transport, (v) influence of membrane transporters, and (vi) comparison of interaction with different segments of the GI tract.

The following two sub-sections present both *in vitro* cell-based methods and *ex vivo* Ussing chambers, summarizing their key attributes and limitations.

5.6.1. In vitro cell-based models

In vitro cell-based studies offer a compromise between *in vitro* studies and *in vivo* studies, by providing intermediary level of complexity, feasibility and some resemblance to the *in vivo* condition. Further, they help in significant reduction in the usage of animals, while still providing information regarding the tested drug molecules. Especially, in the case of monolayers in Transwell® systems which provide an estimation of drug permeability across polarized intestinal cellular monolayer (Pereira et al., 2016).

In vitro cell culture methods are extensively used to evaluate intestinal permeability of drug molecules and/or interaction with the drug delivery systems rapidly. These *in vitro* intestinal cell culture models are developed primarily using immortalized cell lines as the use of primary cell lines have been generally restricted owing to their inability to form polarized epithelial monolayer in *in vitro* conditions (Bohets et al., 2001). There are several types of immortalized cell lines used for this purpose, such as the human colon adenocarcinoma (Caco-2), mucus-producing goblet-like cells (HT29-MTX), Madin-Darby Canine Kidney (MDCK) cells and 2/4/A cells. These cell lines are used either individually or in co-culture to form an *in vitro* intestinal model ranging from simple two-dimensional cell monolayer to three-dimensional model compromising of three or more cell lines. A summary of the different cell-based models reviewed in this section are described in Table 7.

Caco-2 cells are considered as the 'gold standard' and have been extensively used for gathering vast information regarding *in vitro* drug absorption. Caco-2 cells seeded in Transwell® membrane filters differentiate into mature polarized enterocytes, with brush bordered apical side and basolateral side after 19–21 days. Caco-2 monolayers also present the tight junctions between the cells and express several membrane transporters that are present in intestinal epithelia. *In vitro* Caco-2 cell-based monolayers has been used to evaluate intestinal permeability of both small molecule drugs (Mäkilä et al., 2016; Zhang et al., 2012) and large biopharmaceuticals (Li et al., 2014; Shrestha et al., 2018). However, monoculture Caco-2 monolayer models are far from perfect as they lack several important features of intestinal epithelia such as presence of mucus-layer, barrier properties similar to colonic epithelia, poor expression of intestinal enzymes (*e.g.* CYP3A), high variability among clones, and absence of other cell types such as M cells and fibroblasts (Sarmento et al., 2012). To overcome these

Table 7
Characteristics of different intestinal cell models used for assessing the intestinal absorption.

Cell line	Source	Characteristics	References
Caco-2	Human colorectal carcinoma	Differentiation into mature enterocytes with apical and basolateral side; Production of tight junctions; Expression of a wide range of transporters	(Artursson et al., 2001; Buckley et al., 2012; Sarmento et al., 2012)
TC-7	Human, Clone of Caco-2	Morphologically similar to Caco-2; Higher homogeneity and lower variability than Caco-2; High enzyme content and lower P-gp expression; Suitable for passively transported molecules only	(Buckley et al., 2012; Turco et al., 2011)
2/4/A1	Rat	Lower number of active drug transporters and looser tight junctions; 'small intestine-like'; Suitable for passively/paracellularly transported drug molecules	(Tavelin et al., 2003)
MDCK	Canine, dog kidney cell line	Non-intestine and non-human derived cell lines; Comparable to Caco-2 models for drugs using passive transcellular pathways; Low expression of drug transporters	(Bohets et al., 2001; Irvine et al., 1999)
HT29/MTX	Human colorectal carcinoma	Goblet cells; Mucus producing cells	(Araújo and Sarmento, 2013; Lechanteur et al., 2018; Sarmento et al., 2012)
Raji B	Human, B-lymphocytes	Used for conversion of enterocytes to M cells; Used in co-culture with enterocyte like cells.	(Beloqui et al., 2017; Des Rieux et al., 2006)
Caco-2/HT29-MTX	Human colorectal carcinoma	Produces co-culture monolayer with absorptive enterocytes and goblet cells that produces mucus covering the monolayer; Barrier properties can be controlled based on the day of seeding HT29-MTX cells	(Béduneau et al., 2014; Mahler et al., 2009; Wikman-Larhed and Artursson, 1995)
Caco-2/Raji B (FAE)	Human colorectal carcinoma/B-lymphocytes	Resembles human follicle-associated epithelium; Co-culture produces Caco-2 cells with interspersed M-cells; Useful for uptake and internalization studies	(Beloqui et al., 2017; Des Rieux et al., 2006)
Caco-2/HT29-MTX-Raji B	Human colorectal carcinoma/B-lymphocytes	Closely resembles the human intestinal epithelia, with monolayer comprising of three major cells, enterocytes, mucus-producing cell line and M cells; Barrier properties closely resembling small intestine	(Antunes et al., 2013; Araújo and Sarmento, 2013)

limitations, several strategies have been evaluated such as use of sub clones of Caco-2, use of co-culture technique and alternative cell lines.

Owing to the heterogeneity of Caco-2 cell lines, sub clonal cell lines such as TC-7 have been isolated, which have shown to improve homogeneity, and also demonstrate higher enzyme content and lower levels of P-glycoprotein (P-gp) (Pereira-Caro et al., 2010). However, Turco and co-workers have demonstrated that TC-7 cell-based models are suitable only for passively diffusing molecules. On the other hand, TC-7 cells have been regarded as unsuitable for lipophilic and poorly absorbed drugs, and when active transport and/or first pass metabolism is involved (Buckley et al., 2012; Turco et al., 2011). Another alternative cell line used for intestinal permeability is the rat-derived 2/4/A1 cell line. This cell line has lower number of active drug transporters and has looser tight junctions similar which are more similar small intestine compared to colon-like tight junctions in Caco-2 cell lines, thus making it a more promising alternative to Caco-2 monolayer for passively transported drug molecules (Artursson et al., 2001; Tavelin et al., 2003). MDCK cell lines is another alternative, which are derived from dog kidney cells. Despite its non-human origin, these cells are comparable to Caco-2 models for drugs using passive transcellular pathways and exhibit low expression of drug transporters and have negligible metabolic activity (Buckley et al., 2012; Irvine et al., 1999).

In addition to these simple monoculture models, advanced co-culture and triple co-culture models have been developed to incorporate the other important features of intestinal epithelium. Since its establishment in 1995, co-culture of Caco-2 and HT29-MTX has been used significantly. The major characteristics of this model is the presence of mucus layer and reduced TEER values which are more comparable to small intestine values (Araújo and Sarmento, 2013; Mahler et al., 2009). The co-culture model allows evaluation of influence of presence of mucus in drug transport and absorption. Moreover, another study has also demonstrated the influence of the day of the seeding of goblet cells on the barrier properties which includes P-gp expression and paracellular transport (Béduneau et al., 2014).

A co-culture model using Caco-2 and lymphocyte Raji B cells that mimic human follicle associate epithelium (FAE) have also been studied. In the presence of Raji B cells, the enterocytes are converted into M cells, which are a part of mucosal immune system and are mainly found in the FAE of Peyer's patches. M cells are responsible for transcytosic pathway and has the capability to transport wide array of cargos, from drug molecule to therapeutic nanosystems. The successful development of this co-culture is determined by reduced TEER values, increased translocation, loss of microvilli and increased bacterial adherence and translocation (Beloqui et al., 2017; Des Rieux et al., 2006).

To achieve intestinal models that resemble the complexity and closely mimic the *in vivo* condition, a triple cell co-culture model with Caco-2/HT29-MTX/RajiB cells has also been developed. This model combines the advantages of both the abovementioned two co-culture models (Caco-2/HT29-MTX and Caco-2/RajiB) with the presence of mucus layer, decrease TEER levels, and presence of M cells, all of which closely resembles the intestinal epithelia (Antunes et al., 2013; Araújo and Sarmento, 2013). Overall, this system would be a reliable *in vitro* intestinal barrier model to study the absorption of drug molecules alone or encapsulated in drug delivery systems (Sarmento et al., 2012).

5.6.2. *Ex vivo technique: Ussing chambers*

Despite wide application of *in vitro* cellular models, their use is greatly limited by their inability to reflect the complexity and simulate multiple conditions that exists in human intestine. In comparison, the use of functional fresh tissues in *ex vivo* models demonstrate higher degree of interplay among different cell types and closer resemblance of *in vivo* conditions. Some of the extensively used *ex vivo* models used are Ussing chambers, Franz cells, everted intestinal sac method, *etc.* (Pearce et al., 2018; Nunes et al., 2016; Roeselers et al., 2013). In this section, we will mainly discuss the Ussing chambers system as *ex vivo* model to study physiology and permeability across intestinal epithelia.

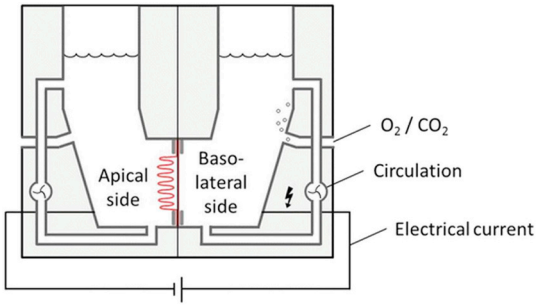

Fig. 7. Schematic representation of Ussing chambers model (Westerhout et al., 2015).

Since its first use in 1950s by Hans Henriksen Ussing, the Ussing chambers model has been used widely for studying physiology, transport of molecules and nanoparticles in a wide variety of epithelial tissues (such as intestine, retinal, reproductive tract, and exocrine/endocrine ducts) and cultured cell lines (Pearce et al., 2018; Kalman and Ussing, 1955; Lundquist and Artursson, 2016; Clarke, 2009). Compared to the original model, several modifications have been made to enhance the performance of the model and numerous alternatives are available (Nunes et al., 2016; Clarke, 2009). One of the major applications of Ussing chambers has been in the field of intestinal physiology and transport, in either rodent tissue or human tissue (Nunes et al., 2016; Lundquist and Artursson, 2016; Clarke, 2009; Westerhout et al., 2014). The use of human intestinal tissues in Ussing chambers experiments have been significantly augmented owing to the increase in the number of bariatric surgeries during which intestine tissue samples can be donated without any safety problems to the patient (Ng et al., 2014). Nonetheless, the tissues obtained from patients who are obese or suffering from colorectal cancer differ from normal donors, and further the tissue transport has to be fast to minimize tissue deterioration (Lundquist and Artursson, 2016).

A schematic representation of the Ussing chambers is shown in Fig. 7 (Westerhout et al., 2015). The Ussing chambers system mainly comprises of chambers that are filled with nutrient rich buffer (commonly, Krebs Ringer buffer), and with a freshly excised and flattened tissue that is positioned in the window between the two adjacent chambers. The tissue is placed as such the mucosal side is facing one chamber (referred as luminal side or apical), and the serosal side is facing the second chamber (also known as basolateral side). The test samples or formulations are placed on the apical side of the system (Nunes et al., 2016; Lundquist and Artursson, 2016). Oxygen and carbon dioxide (Carbogen gas for mammalian physiological buffers) are continuously supplied to the system, which has additional benefit of maintain the physiological pH 7.4 and maintaining minimum level of unstirred layer. Both chambers are water-jacketed to maintain the temperature at 37 °C (Clarke, 2009; Buckley et al., 2012). The chambers can be either vertical or horizontal, and the electrophysiological properties of the tissue are continuously monitored (Clarke, 2009). Based on the time-concentration data obtained during the experiment, apparent permeability of the drug can be estimated (Sjöberg et al., 2013). In addition to commonly used buffers, several studies have demonstrated the possibility of using simulated fluids to more closely resemble the *in vivo* conditions (Wuyts et al., 2015). Nonetheless the intestinal integrity and viability must be continuously monitored and can be measured by two electrodes in the two chambers, that measures the TEER, potential difference and the short-circuit current (Sjöberg et al., 2013; Lautenschläger et al., 2013). Additionally, markers such as Lucifer yellow, lactate dehydrogenase release, and histological examinations can also be performed to ensure the viability of the tissue (Nunes et al., 2016; Westerhout et al., 2015; Rozehnal et al., 2012).

Ussing chambers have been extensively used to study the permeability of drug molecules in free form or encapsulated in nanosystems,

across animal or human intestinal tissues (Sjöberg et al., 2013; Rozehnal et al., 2012). This model can serve as a critical tool for evaluating the interaction of nanoparticles with the intestinal epithelial tissue in a more complex scenario as compared to cell-based models (Lautenschläger et al., 2013). Several studies have used Ussing chambers model to evaluate and understand the behavior of small molecule drugs encapsulated in delivery systems. A recent study involved the evaluation of carbamazepine permeability from lipid-polymer hybrid nanoparticles in mice jejunal sections in Ussing chambers model (Ana et al., 2019). Another study by Barbieri et al. (2015) evaluated the permeability of anti-cancer drug tamoxifen from lecithin-chitosan nanoparticles. Freshly excised rat jejunal tissues in Ussing chambers were used to demonstrate the permeation enhancing effect of the nanoparticles as compared to the free drug suspension (Barbieri et al., 2015). Overall, these studies demonstrate Ussing chambers model as a feasible model to assess the permeability of drugs from advanced drug delivery systems.

Ussing chambers system allows the measurement of intestinal physiology and transport across intact polarized intestinal tissue for a limited period and involves more complexity of the barrier when compared to cell-based culture models. The model also allows bidirectional drug transport and as well as the evaluation of the influence of regional intestinal differences and species differences in drug transport mechanism. Moreover, the influence of drug transporters expressed on the intestinal tissues can also be assessed using this model (Nunes et al., 2016; Clarke, 2009; Sjöberg et al., 2013; Fortuna et al., 2012). Despite such advantages, Ussing chambers model is a labor-intensive process and do not entirely mimic the *in vivo* situation, as it does not include circulation and lymphatic drainage systems thereby limiting its application to < 150–180 min (Lundquist and Artursson, 2016; Westerhout et al., 2015; Sjöberg et al., 2013). Furthermore, during the experiment period the viability and the integrity of the tissue must be maintained. Additionally, the overall process is time-consuming and there could be possible damage or morphological changes during the tissue preparation, mounting or removal (Nunes et al., 2016).

6. Unmet technological needs and future prospects

6.1. Limitations of the available formulation-related technologies

Limitations from a technological viewpoint can be identified regarding formulation design as well as analytical technologies. It makes sense to further consider limitations of silico tools and any other information technology that could guide formulation development. Cheminformatics has in the drug discovery phase much contributed to a better integration of otherwise fragmented information gathered across the relevant disciplines (Lawless et al., 2016). Especially machine learning and artificial intelligence are increasingly used in cheminformatics to guide the selection of suitable drug candidates (Lo et al., 2018). Compared to such approaches to obtain a viable drug candidate, the development of the drug product is still guided mostly by expert knowledge. Flow charts are given in the pharmaceutical literature to aid with the selection of a suitable formulation approach for a new active compound by addressing the individual physicochemical and biopharmaceutical characteristics (Kuentz et al., 2016). However, with an increasing flood of information coming from different computational simulations (Section 3), *in vitro* methods (Section 4) as well as *in vivo* experiments, it becomes difficult to keep up with informed decision making about formulation and process technique. Formulation development is therefore encouraged to increasingly use modern algorithmic tools and there is much to learn from the drug discovery phase (Smith et al., 2018). Just one example is whether or not multitask deep learning is practical in pharma applications (Ramsundar et al., 2017). Since evaluation of such algorithms requires rather large datasets, a testing in drug discovery is so far much easier than in drug product development. Further hurdles are in the present case both software difficulties and lack of understanding of how robust such multitask deep learning networks are. A possible solution is to provide high quality open source software programs that can be used broadly in the pharmaceutical industry (Ramsundar et al., 2017). Thus, what is currently missing is evaluation studies of such modern computational approaches in the field of drug product designs as well as manufacturing.

Another gap regarding formulation development is to have a broader range of pharmaceutical materials and excipients to select from. Many new concepts emerge from modern drug delivery and targeting but they are hard to implement later in formulation development based on the existing range of pharmaceutical additives (Tibbitt et al., 2016). However, innovative excipients that are novel chemical entities would have to overcome diverse toxicological as well as regulatory hurdles in pharmaceutical development (Elder et al., 2016). Therefore, much excipient novelty has been based rather on simple co-processing of known excipients to enhance processability in manufacturing such as to enhance tableting performance (Rojas et al., 2012). An interesting idea is to harness molecularly designed interactions between additives and polymers to obtain, for example, novel matrices for solid drug dispersions, which demonstrate distinct advantages over a simple physical mixture of the components (Ditzinger et al., 2019). The topic of novel materials/excipients has to be addressed more extensively in pharmaceutics to bring good novel drug delivery ideas to a formulation technology that is viable on the market. The given technical possibilities of novel materials/excipients have to be aligned with the needs identified from oral delivery challenges. Such biopharmaceutical requirements must be clarified based on proper understanding of how formulations are intraluminally processed. The state-of-the-art methods that were outlined in Section 5 therefore provide critical information on what is needed from an oral delivery perspective. This should lead to targeted characteristics of any novel excipients and their technical and regulatory hurdles must be addressed to bring novel drug delivery ideas to a viable formulation technology that can be introduced to the pharmaceutical market.

Unmet technological needs are not only about market formulations but are also given considering preclinical and clinical delivery systems that are essential to develop drug products. There are, for example, limitations in process technologies to obtain desired colloidal drug delivery systems. Such colloidal solutions are mostly used as preclinical or clinical oral formulations, but they also serve as intermediate products to obtain a final solid dosage form. Much innovation is expected from microfluidic devices as they can handle liquids at the nanoliter scale with advantages of process intensification (Mitic and Gernaey, 2016) and facilitated scale-up by multiplying the microscale circuits to the needs of production. Microfluidics has been applied to prepare liposomes, lipid nanoparticles, polymeric nanoparticles, and other hybrid nanoparticles (Garg et al., 2016).

Diverse limitations of formulation design and logistics may be addressed by another emerging technology that is about 3D printing of drug products. This umbrella term actually includes different manufacturing techniques, which have basic elements in common. Thus, material is deposited in digital controlled manner to produce a layer-by-layer structure that can take any geometry of choice. This solid freedom fabrication has been classified into printing based on inkjet systems, nozzle-based deposition systems, and laser-based writing systems (Goole and Amighi, 2016). To date, technologies based on 3D printing techniques have been implemented for clinical use of printed medical products such as implants and there is currently much emphasis on printing drug products (Awad et al., 2018). Spurred by the first FDA approval of a pharmaceutical product (SPRITAM®), the 3D printing technology has gained momentum in pharmaceutics (Khatri et al., 2018). There are obvious advantages of this approach to cope with needs of increased product complexity, personalization, and on-demand manufacturing (Norman et al., 2017). However, is it really a disruptive innovation for pharmaceutical manufacturing in general or will it rather take in the future only niche applications in personalized drug

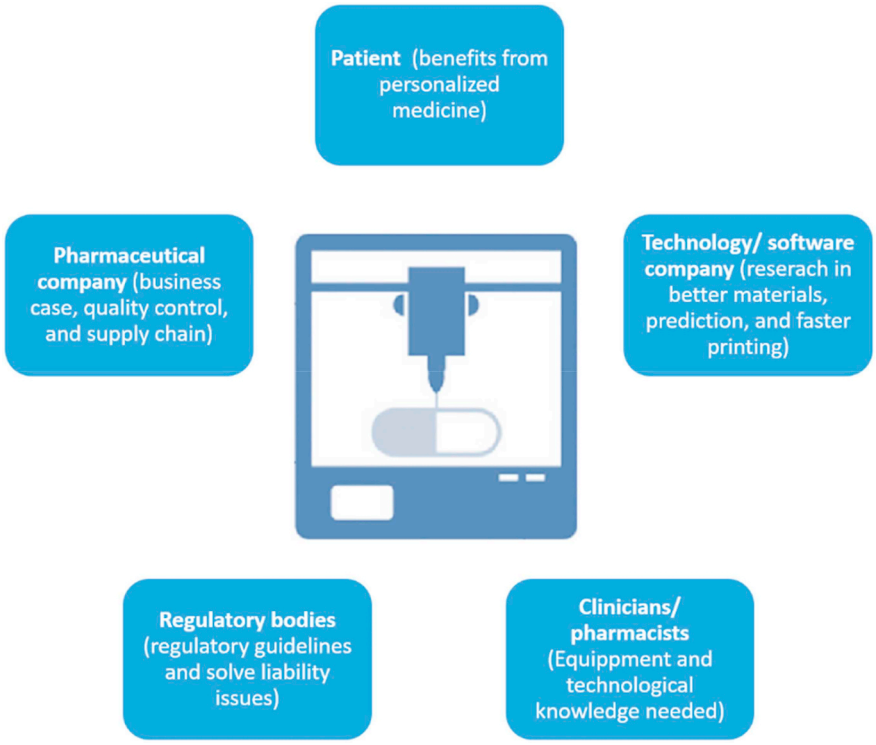

Fig. 8. Stakeholders and key issues in the field of 3D printed drug products as modified from Liang et al. (2019).

products? Fig. 8 displays stake holders as well as key issues of 3D printing that have to be addressed in the following years (Liang et al., 2019). There is in particular a regulatory framework needed and aspects of quality control must be clarified when a community pharmacy is printing drug products as personalized medicine. Since printing in a pharmacy or a hospital is based on the contribution of the drug manufacturer, software developer, and a pharmacist, it must be clarified who is ultimately liable in case of any product failures. The true potential of drug product 3D printing is therefore depending on how the named key issues are addressed and solved over the coming years.

A further gap to overcome can be seen in the field of real-time analytics. There is a broad range of process analytics and research into novel dip probes or flow-through cells is ongoing (Simon et al., 2015). Process analytical technologies have on the one hand relevance for manufacture of pharmaceutical dosage forms (Rantanen and Khinast, 2015) but there is further usage that is of particular interest from a biopharmaceutics perspective. Thus, real-time analytics is increasingly used to obtain dynamic information from *in vitro* testing. Immersion probes or flow-through cells are options to make use of, for example, UV or vibrational spectroscopy, or by employing different sensors of particle analysis (Kuentz, 2014). Analytical probes can hence aid with closing the gaps addressed previously (in Section 5) on drug assessment under biorelevant conditions and a prominent example is to use UV immersion probes to assess drug supersaturation (Palmelund et al., 2016). While UV dip probes are a relatively established technique, there are novel process analyzers needed especially, when trying to cope with complex media in simulated intestinal fluids. Formulation processing in the intestine leads to dynamic changes of droplets, particles, and colloids emerging from pharmaceutical formulations. Apart from the challenges to simulate these processes *in vitro*, it will be important to also monitor changes in a dynamic way. Sensor probes should here not only measure drug concentrations and optional supersaturation, but also structural changes should be monitored and a challenging example is digestion of LBF (Vithani et al., 2017; Kuentz, 2018). Such complex media typically exhibit relatively high turbidity that makes it hard to measure by means of any optical spectroscopy. Thus, especially promising dip sensors for such complex digested formulations are potentiometric probes that can measure free drug concentrations of ionizable compounds (Tran et al., 2018). Measurement free drug concentrations in real-time would be highly desirable in any *in vitro* test because drug activity is driving the absorptive flux across the intestinal wall. A finer monitoring of concentration changes by adequate real-time analytics further help with a better understanding and ultimately with computational modelling of how drug delivery systems are processed in the GI tract.

In summary, the design of oral formulations should be based on data from *in silico*, *in vitro*, and later *in vivo* data. Limitations are not just given on the available types of materials, delivery systems, and manufacturing techniques but also the limitations of computational and analytical methods must be addressed in the context of oral formulation design. Advancement of these formulation-related technologies will be needed to address the still unmet needs of modern oral drug delivery.

6.2. Research strategies: a change of paradigm is needed

In prolongation of the above said, the assessment of drugs and development of formulations regarding their probable biopharmaceutical behavior the following strategies are proposed:

1) For the screening of new drug entities, a classical *in vitro* solubility test and permeation test from solutions (both using artificial barriers and cell-based assays) still appears suitable to classify the compounds according to the expected kind and degree of problems in their formulation and to choose a promising formulation strategy.
2) The use of biorelevant media to assess their interaction with the main components of the fluids is also encouraged both for solubility and for permeation.
3) However, for absorption (permeation) assessment and biopharmaceutical performance prediction of formulations, the concept of freely dissolved drug molecules as being the key factor should be appreciated. The consequent use of a clear nomenclature that distinguishes the apparently and molecularly dissolved fractions of the

drug is needed.

4) Free drug concentrations evolving after dispersion of the respective drug formulation in biorelevant set-ups, possibly including lipolysis for LBFs, may be quantified by dialysis methods. However, a permeation experiment from such formulation dispersion may in a pragmatic way include the concept of free drug concentration within the permeation assay.
5) Such approach according to steps 1) to 3) still does not take into account the dynamic changes occurring during the GI passage of oral dosage forms, including dilution, changes of composition of the digestive fluids, concentration changes due to absorption or metabolism, *etc.* Combined dissolution/permeation approaches may provide an experimental access to mimic such changes.
6) For detailed mechanistic studies, the investigation of the kinetics of all the above named inter-related processes should take into account, including the active transport, metabolism *etc.* The models will become increasingly complex according to increasing need for better understanding and better modelling.

Therefore, for the time being, animal experiments are still needed to validate the intraluminal behavior of drug from advanced drug delivery systems.

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References

Adeoye, O., Cabral-Marques, H., 2017. Cyclodextrin nanosystems in oral drug delivery: a mini review. Int. J. Pharm. 531 (2), 521–531.
Agatemor, C., Ibsen, K.N., Tanner, E.E.L., Mitragotri, S., 2018. Ionic liquids for addressing unmet needs in healthcare. Bioengineering & Translational Medicine 3 (1), 7–25.
Allen, A., Pearson, J.P., Hutton, D.A., Mall, A.H., Coan, R.M., Sellers, L.A., 1989. The dependence of size and functional properties of pig gastric mucus on the isolation method used. Symp. Soc. Exp. Biol. 43, 241–248.
Amidon, G.L., Lennernaes, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12 (3), 413–420.
Ana, R., Mendes, M., Sousa, J., Pais, A., Falcão, A., Fortuna, A., Vitorino, C., 2019. Rethinking carbamazepine oral delivery using polymer-lipid hybrid nanoparticles. Int. J. Pharm. 554, 352–365.
Anby, M.U., Williams, H.D., McIntosh, M., Benameur, H., Edwards, G.A., Pouton, C.W., Porter, C.J.H., 2012. Lipid digestion as a trigger for supersaturation: evaluation of the impact of supersaturation stabilization on the in vitro and in vivo performance of self-emulsifying drug delivery systems. Mol. Pharm. 9 (7), 2063–2079.
Anby, M.U., Nguyen, T., Yeap, Y.Y., Feeney, O.M., Williams, H.D., Benameur, H., Pouton, C.W., Porter, C.J.H., 2014. An in vitro digestion test that reflects rat intestinal conditions to probe the importance of formulation digestion vs first pass metabolism in danazol bioavailability from lipid based formulations. Mol. Pharm. 11, 4069–4083.
Anderberg, E.K., Lindmark, T., Artursson, P., 1993. Sodium caprate elicits dilatations in human intestinal tight junctions and enhances drug absorption by the paracellular route. Pharm. Res. 10 (6), 857–864.
Anderson, B.D., Flora, K.P., 1996. Preparation of water-soluble compounds through salt formation. In: Wermuth, C.G. (Ed.), The Practice of Medicinal Chemistry. Academic Press, London, pp. 739–754.
Andreas, C.J., Rosenberger, J., Butler, J., Augustijns, P., McAllister, M., Abrahamsson, B., Dressman, J., 2018. Introduction to the OrBiTo decision tree to select the most appropriate in vitro methodology for release testing of solid oral dosage forms during development. Eur. J. Pharm. Biopharm. 130, 207–213.
Antoine, D., Pellequer, Y., Tempesta, C., Lorscheidt, S., Kettel, B., Tamadon, L., Jannin, V., Demarne, F., Lamprecht, A., Béduneau, A., 2015. Biorelevant media resistant co-culture model mimicking permeability of human intestine. Int. J. Pharm. 481 (1–2), 27–36.
Antunes, F., Andrade, F., Araújo, F., Ferreira, D., Sarmento, B., 2013. Establishment of a triple co-culture in vitro cell models to study intestinal absorption of peptide drugs. Eur. J. Pharm. Biopharm. 83 (3), 427–435.
Araújo, F., Sarmento, B., 2013. Towards the characterization of an in vitro triple co-culture intestine cell model for permeability studies. Int. J. Pharm. 458 (1), 128–134.
Artursson, P., Palm, K., Luthman, K., 2001. Caco-2 monolayers in experimental and theoretical predictions of drug transport. Adv. Drug Deliv. Rev. 46 (1–3), 27–43.
Augustijns, P., Brewster, M.E., 2012. Supersaturating drug delivery systems: fast is not necessarily good enough. J. Pharm. Sci. 101 (1), 7–9.
Augustijns, P., Wuyts, B., Hens, B., Annaert, P., Butler, J., Brouwers, J., 2014. A review of drug solubility in human intestinal fluids: implications for the prediction of oral absorption. Eur. J. Pharm. Sci. 57, 322–332.
Awad, A., Trenfield, S.J., Gaisford, S., Basit, A.W., 2018. 3D printed medicines: a new branch of digital healthcare. Int. J. Pharm. 548, 586–596.
Bakala-N'Goma, J.-C., Williams, H.D., Sassene, P.J., Kleberg, K., Calderone, M., Jannin, V., Igonin, A., Partheil, A., Marchaud, D., Jule, E., et al., 2015. Toward the establishment of standardized in vitro tests for lipid-based formulations. 5. Lipolysis of representative formulations by gastric lipase. Pharm. Res. 32 (4), 1279–1287.
Barbieri, S., Buttini, F., Rossi, A., Bettini, R., Colombo, P., Ponchel, G., Sonvico, F., Colombo, G., 2015. Ex vivo permeation of tamoxifen and its 4-OH metabolite through rat intestine from lecithin/chitosan nanoparticles. Int. J. Pharm. 491 (1), 99–104.
Basit, A.W., McConnell, E.L., 2011. Drug delivery to the colon. In: Wilson, C.G., Crowley, P.J. (Eds.), Controlled Release in Oral Drug Delivery, pp. 385–399.
Béduneau, A., Tempesta, C., Fimbel, S., Pellequer, Y., Jannin, V., Demarne, F., Lamprecht, A., 2014. A tunable Caco-2/HT29-MTX co-culture model mimicking variable permeabilities of the human intestine obtained by an original seeding procedure. Eur. J. Pharm. Biopharm. 87 (2), 290–298.
Beloqui, A., Brayden, D.J., Artursson, P., Préat, V., Des Rieux, A., 2017. A human intestinal M-cell-like model for investigating particle, antigen and microorganism translocation. Nat. Protoc. 12 (7), 1387–1399.
Benson, S.P., Pleiss, J., 2014. Molecular dynamics simulations of self-emulsifying drug-delivery systems (SEDDS): influence of excipients on droplet nanostructure and drug localization. Langmuir 30 (28), 8471–8480.
Berben, P., Mols, R., Brouwers, J., Tack, J., Augustijns, P., 2017. Gastrointestinal behavior of itraconazole in humans - part 2: the effect of intraluminal dilution on the performance of a cyclodextrin-based solution. Int. J. Pharm. 526 (1–2), 235–243.
Berben, P., Bauer-Brandl, A., Brandl, M., Faller, B., Flaten, G.E., Jacobsen, A.-C., Brouwers, J., Augustijns, P., 2018a. Drug permeability profiling using cell-free permeation tools: overview and applications. Eur. J. Pharm. Sci. 119, 219–233.
Berben, P., Brouwers, J., Augustijns, P., 2018b. The artificial membrane insert system as predictive tool for formulation performance evaluation. Int. J. Pharm. 537 (1–2), 22–29.
Bergström, C.A.S., Larsson, P., 2018. Computational prediction of drug solubility in water-based systems: qualitative and quantitative approaches used in the current drug discovery and development setting. Int. J. Pharm. 540 (1–2), 185–193.
Bergström, C.A.S., Wassvik, C.M., Johansson, K., Hubatsch, I., 2007. Poorly soluble marketed drugs display solvation limited solubility. J. Med. Chem. 50 (23), 5858–5862.
Bergström, C.A.S., Charman, W.N., Porter, C.J.H., 2016. Computational prediction of formulation strategies for beyond-rule-of-5 compounds. Adv. Drug Deliv. Rev. 101, 6–21.
Bermejo, M., Paixão, P., Hens, B., Tsume, Y., Koenigsknecht, M.J., Baker, J.R., Hasler, W.L., Lionberger, R., Fan, J., Dickens, J., et al., 2018. Linking the gastrointestinal behavior of ibuprofen with the systemic exposure between and within humans-part 1: fasted state conditions. Mol. Pharm. 15 (12), 5454–5467.
Bernardi, R.C., Melo, M.C.R., Schulten, K., 2015. Enhanced sampling techniques in molecular dynamics simulations of biological systems. Biochim. Biophys. Acta 1850 (5), 872–877.
Berry, D.J., Steed, J.W., 2017. Pharmaceutical cocrystals, salts and multicomponent systems; intermolecular interactions and property based design. Adv. Drug Deliv. Rev. 117, 3–24.
Berthelsen, R., Sjögren, E., Jacobsen, J., Kristensen, J., Holm, R., Abrahamsson, B., Müllertz, A., 2014. Combining in vitro and in silico methods for better prediction of surfactant effects on the absorption of poorly water soluble drugs - a fenofibrate case example. Int. J. Pharm. 473 (1), 356–365.
Bhat, P.A., Dar, A.A., Rather, G.M., 2008. Solubilization capabilities of some cationic, anionic, and nonionic surfactants toward the poorly water-soluble antibiotic drug erythromycin. J. Chem. Eng. Data 53 (6), 1271–1277.
Bibi, H.A., Di Cagno, M., Holm, R., Bauer-Brandl, A., 2015. Permeapad™ for investigation of passive drug permeability: the effect of surfactants, co-solvents and simulated intestinal fluids (FaSSIF and FeSSIF). Int. J. Pharm. 493 (1–2), 192–197.
Bibi, H.A., Holm, R., Bauer-Brandl, A., 2017. Simultaneous lipolysis/permeation in vitro model, for the estimation of bioavailability of lipid based drug delivery systems. Eur. J. Pharm. Biopharm. 117, 300–307.
Birru, W.A., Warren, D.B., Han, S., Benameur, H., Porter, C.J.H., Pouton, C.W., Chalmers, D.K., 2017a. Computational models of the gastrointestinal environment. 2. phase behavior and drug solubilization capacity of a type I lipid-based drug formulation after digestion. Mol. Pharm. 14 (3), 580–592.
Birru, W.A., Warren, D.B., Headey, S.J., Benameur, H., Porter, C.J.H., Pouton, C.W., Chalmers, D.K., 2017b. Computational models of the gastrointestinal environment. 1. The effect of digestion on the phase behavior of intestinal fluids. Mol. Pharm. 14 (3), 566–579.
Bodor, N., 1984. Techniques of solubilization of drugs. (drugs and the pharmaceutical sciences series, Vol 12.). J. Pharm. Sci. 73 (2), 288.
Bogardus, J.B., Blackwood, R.K., 1979. Solubility of doxycycline in aqueous solution. J. Pharm. Sci. 68, 188–194.
Bohets, H., Annaert, P., Mannens, G., van Beijsterveldt, L., Anciaux, K., Verboven, P., Meuldermans, W., Lavrijsen, K., 2001. Strategies for absorption screening in drug discovery and development. Curr. Top. Med. Chem. 1 (5), 367–383.
Borbás, E., Nagy, Z.K., Nagy, B., Balogh, A., Farkas, B., Tsinman, O., Tsinman, K., Sinkó, B., 2018. The effect of formulation additives on in vitro dissolution-absorption profile and in vivo bioavailability of telmisartan from brand and generic formulations. Eur. J. Pharm. Sci. 114, 310–317.
Braybrooks, M.P., Barry, B.W., Abbs, E.T., 1975. The effect of mucin on the bioavailability of tetracycline from the gastrointestinal tract; in vivo, in vitro correlations. J. Pharm. Pharmacol. 27 (7), 508–515.
Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunçao, R., Balance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., et al., 2019. INFOGEST static in vitro simulation of gastrointestinal food digestion. Nat. Protoc. https://doi.org/10.1038/s41596-018-0119-1.
Brouwers, J., Augustijns, P., 2014. Resolving intraluminal drug and formulation behavior: gastrointestinal concentration profiling in humans. Eur. J. Pharm. Sci. 61, 2–10.
Brouwers, J., Tack, J., Lammert, F., Augustijns, P., 2006. Intraluminal drug and

formulation behavior and integration in in vitro permeability estimation: a case study with amprenavir. J. Pharm. Sci. 95 (2), 372–383.
Brouwers, J., Tack, J., Augustijns, P., 2007. Parallel monitoring of plasma and intraluminal drug concentrations in man after oral administration of fosamprenavir in the fasted and fed state. Pharm. Res. 24 (10), 1862–1869.
Brouwers, J., Brewster, M.E., Augustijns, P., 2009. Supersaturating drug delivery systems: the answer to solubility-limited oral bioavailability? J. Pharm. Sci. 98 (8), 2549–2572.
Brouwers, J., Geboers, S., Mols, R., Tack, J., Augustijns, P., 2017. Gastrointestinal behavior of itraconazole in humans - part 1: supersaturation from a solid dispersion and a cyclodextrin-based solution. Int. J. Pharm. 525 (1), 211–217.
Brouwers, J., Van Den Abeele, J., Augustijns, P. 2018. Unraveling the fate of oral drug products in the human GI tract. https://www.aapsnewsmagazine.org/articles/2018/dec18/cover-story-dec18
Buckley, S.T., Fischer, S.M., Fricker, G., Brandl, M., 2012. In vitro models to evaluate the permeability of poorly soluble drug entities: challenges and perspectives. Eur. J. Pharm. Sci. 45 (3), 235–250.
Buckley, S.T., Frank, K.J., Fricker, G., Brandl, M., 2013. Biopharmaceutical classification of poorly soluble drugs with respect to “enabling formulations”. Eur. J. Pharm. Sci. 50 (1), 8–16.
Butler, J.M., Dressman, J.B., 2010. The developability classification system: application of biopharmaceutics concepts to formulation development. J. Pharm. Sci. 99 (12), 4940–4954.
Capolino, P., Guérin, C., Paume, J., Giallo, J., Ballester, J.M., Cavalier, J.F., Carrière, F., 2011. In vitro gastrointestinal lipolysis: replacement of human digestive lipases by a combination of rabbit gastric and porcine pancreatic extracts. Food digestion 2, 43–51.
Chamieh, J., Merdassi, H., Rossi, J.-C., Jannin, V., Demarne, F., Cottet, H., 2017. Size characterization of lipid-based self-emulsifying pharmaceutical excipients during lipolysis using Taylor dispersion analysis with fluorescence detection. Int. J. Pharm. 537 (1–2), 94–101.
Chen, J., Ormes, J.D., Higgins, J.D., Taylor, L.S., 2015. Impact of surfactants on the crystallization of aqueous suspensions of celecoxib amorphous solid dispersion spray dried particles. Mol. Pharm. 12 (2), 533–541.
Clarke, L.L., 2009. A guide to Ussing chamber studies of mouse intestine. American journal of physiology-gastrointestinal and liver physiology 296 (6), G1151–G1166.
Crocker, J.C., Grier, D.G., 1996. Methods of digital video microscopy for colloidal studies. J. Colloid Interface Sci. 179 (1), 298–310.
Crowther, R.S., Marriott, C., 1984. Counter-ion binding to mucus glycoproteins. J. Pharm. Pharmacol. 36 (1), 21–26.
Cuiné, J.F., Charman, W.N., Pouton, C.W., Edwards, G.A., Porter, C.J.H., 2007. Increasing the proportional content of surfactant (Cremophor EL) relative to lipid in self-emulsifying lipid-based formulations of danazol reduces oral bioavailability in beagle dogs. Pharm. Res. 24 (4), 748–757.
Dahan, A., Hoffman, A., 2007. The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats. Eur. J. Pharm. Biopharm. 67 (1), 96–105.
Dengale, S.J., Hussen, S.S., Krishna, B., Musmade, P.B., Shenoy, G.G., Bhat, K., 2015. Fabrication, solid state characterization and bioavailability assessment of stable binary amorphous phases of Ritonavir with Quercetin. Eur. J. Pharm. Biopharm. 89, 329–338.
Des Rieux, A., Fievez, V., Garinot, M., Schneider, Y.-J., Préat, V., 2006. Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. J. Control. Release 116 (1), 1–27.
Desai, M.A., Mutlu, M., Vadgama, P., 1992. A study of macromolecular diffusion through native porcine mucus. Experientia 48 (1), 22–26.
Descamps, M., Willart, J.F., 2016. Perspectives on the amorphisation/milling relationship in pharmaceutical materials. Adv. Drug Deliv. Rev. 100, 51–66.
Di Cagno, M.P., Bauer-Brandl, A., 2016. Assembly for Assessing Drug Permeability With Adjustable Biomimetic Properties, United States Patent Application. (20180031465).
Di Cagno, M., Bibi, H.A., Bauer-Brandl, A., 2015. New biomimetic barrier Permeapad™ for efficient investigation of passive permeability of drugs. Eur. J. Pharm. Sci. 73, 29–34.
Dill, W.A., Kazenko, A., Wolf, L.M., Glazko, A.J., 1956. Studies on 5,5′-diphenylhydantoin (Dilantin) in animals and man. J. Pharmacol. Exp. Ther. 118, 270–279.
Ditzinger, F., Scherer, U., Schönenberger, M., Holm, R., Kuentz, M., 2019. Modified polymer matrix in pharmaceutical hot melt extrusion by molecular interactions with a carboxylic co-former. Mol. Pharm. 16 (1), 141–150.
Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm. Res. 15 (1), 11–22.
Edueng, K., Mahlin, D., Larsson, P., Bergström, C.A.S., 2017. Mechanism-based selection of stabilization strategy for amorphous formulations: insights into crystallization pathways. J. Control. Release 256, 193–202.
Elder, D.P., Holm, R., Lopez de Diego, H., 2013. Use of pharmaceutical salts and co-crystals to address the issue of poor solubility. Int. J. Pharm. 453 (1), 88–100.
Elder, D.P., Kuentz, M., Holm, R., 2016. Pharmaceutical excipients – quality, regulatory and biopharmceutical considerations. Eur. J. Pharm. Sci. 87, 88–99.
Elvang, P.A., Hinna, A.H., Brouwers, J., Hens, B., Augustijns, P., Brandl, M., 2016. Bile salt micelles and phospholipid vesicles present in simulated and human intestinal fluids: structural analysis by flow field-flow fractionation/multiangle laser light scattering. J. Pharm. Sci. 105 (9), 2832–2839.
Elvang, P.A., Jacobsen, A.-C., Bauer-Brandl, A., Stein, P.C., Brandl, M., 2018. Co-existing colloidal phases in artificial intestinal fluids assessed by AF4/MALLS and DLS: a systematic study into cholate & (lyso-) phospholipid blends, incorporating celecoxib as a model drug. Eur. J. Pharm. Sci. 120, 61–72.
Fabiano, A., Zambito, Y., Bernkop-Schnürch, A., 2017. About the impact of water movement on the permeation behaviour of nanoparticles in mucus. Int. J. Pharm. 517 (1–2), 279–285.
Fagerberg, J.H., Bergström, C.A.S., 2015. Intestinal solubility and absorption of poorly water soluble compounds: predictions, challenges and solutions. Ther. Deliv. 6 (8), 935–959.
Fagerberg, J.H., Tsinman, O., Sun, N., Tsinman, K., Avdeef, A., Bergström, C.A.S., 2010. Dissolution rate and apparent solubility of poorly soluble drugs in biorelevant dissolution media. Mol. Pharm. 7 (5), 1419–1430.
Fagerberg, J.H., Karlsson, E., Ulander, J., Hanisch, G., Bergström, C.A.S., 2015. Computational prediction of drug solubility in fasted simulated and aspirated human intestinal fluid. Pharm. Res. 32 (2), 578–589.
Feeney, O.M., Crum, M.F., McEvoy, C.L., Trevaskis, N.L., Williams, H.D., Pouton, C.W., Charman, W.N., Bergström, C.A.S., Porter, C.J.H., 2016. 50years of oral lipid-based formulations: provenance, progress and future perspectives. Adv. Drug Deliv. Rev. 101, 167–194.
Feng, F., Peng, T., Huang, Z., Singh, V., Shi, Y., Wen, T., Lu, M., Quan, G., Pan, X., Wu, C., 2018. Polymer–surfactant system based amorphous solid dispersion: precipitation inhibition and bioavailability enhancement of itraconazole. Pharmaceutics 10 (2), 53. https://doi.org/10.3390/pharmaceutics10020053.
Fernandez, S., Jannin, V., Rodier, J.-D., Ritter, N., Mahler, B., Carrière, F., 2007. Comparative study on digestive lipase activities on the self emulsifying excipient Labrasol, medium chain glycerides and PEG esters. Biochim. Biophys. Acta 1771 (5), 633–640.
Fernandez, S., Rodier, J.-D., Ritter, N., Mahler, B., Demarne, F., Carrière, F., Jannin, V., 2008. Lipolysis of the semi-solid self-emulsifying excipient Gelucire 44/14 by digestive lipases. Biochim. Biophys. Acta 1781 (8), 367–375.
Fernandez, S., Chevrier, S., Ritter, N., Mahler, B., Demarne, F., Carrière, F., Jannin, V., 2009. In vitro gastrointestinal lipolysis of four formulations of piroxicam and cinnarizine with the self emulsifying excipients Labrasol and Gelucire 44/14. Pharm. Res. 26 (8), 1901–1910.
Fernandez, S., Jannin, V., Chevrier, S., Chavant, Y., Demarne, F., Carrière, F., 2013. In vitro digestion of the self-emulsifying lipid excipient Labrasol(®) by gastrointestinal lipases and influence of its colloidal structure on lipolysis rate. Pharm. Res. 30 (12), 3077–3087.
Flaten, G.E., Bunjes, H., Luthman, K., Brandl, M., 2006a. Drug permeability across a phospholipid vesicle-based barrier 2. Characterization of barrier structure, storage stability and stability towards pH changes. Eur. J. Pharm. Sci. 28 (4), 336–343.
Flaten, G.E., Dhanikula, A.B., Luthman, K., Brandl, M., 2006b. Drug permeability across a phospholipid vesicle based barrier: a novel approach for studying passive diffusion. Eur. J. Pharm. Sci. 27 (1), 80–90.
Fong, S.Y.K., Bauer-Brandl, A., Brandl, M., 2017a. Oral bioavailability enhancement through supersaturation: an update and meta-analysis. Expert opinion on drug delivery 14 (3), 403–426.
Fong, S.Y.K., Poulsen, J., Brandl, M., Bauer-Brandl, A., 2017b. A novel microdialysis-dissolution/permeation system for testing oral dosage forms: a proof-of-concept study. Eur. J. Pharm. Sci. 96, 154–163.
Fortuna, A., Alves, G., Falcao, A.C., Soares da Silva, P., 2012. Evaluation of the permeability and P-glycoprotein efflux of carbamazepine and several derivatives across mouse small intestine by the Ussing chamber technique. Epilepsia 53 (3), 529–538.
Frank, K.J., Rosenblatt, K.M., Westedt, U., Hölig, P., Rosenberg, J., Mägerlein, M., Fricker, G., Brandl, M., 2012a. Amorphous solid dispersion enhances permeation of poorly soluble ABT-102: true supersaturation vs. apparent solubility enhancement. Int. J. Pharm. 437 (1–2), 288–293.
Frank, K.J., Westedt, U., Rosenblatt, K.M., Hölig, P., Rosenberg, J., Mägerlein, M., Fricker, G., Brandl, M., 2012b. The amorphous solid dispersion of the poorly soluble ABT-102 forms nano/microparticulate structures in aqueous medium: impact on solubility. Int. J. Nanomedicine 7, 5757–5768.
Friedl, H., Dünnhaupt, S., Hintzen, F., Waldner, C., Parikh, S., Pearson, J.P., Wilcox, M.D., Bernkop-Schnürch, A., 2013. Development and evaluation of a novel mucus diffusion test system approved by self-nanoemulsifying drug delivery systems. J. Pharm. Sci. 102 (12), 4406–4413.
Friesen, D.T., Shanker, R., Crew, M., Smithey, D.T., Curatolo, W.J., Nightingale, J.A.S., 2008. Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview. Mol. Pharm. 5 (6), 1003–1019.
Ganesan, A., Coote, M.L., Barakat, K., 2017. Molecular dynamics-driven drug discovery: leaping forward with confidence. Drug Discov. Today 22 (2), 249–269.
Gantzsch, S.P., Kann, B., Ofer-Glaessgen, M., Loos, P., Berchtold, H., Balbach, S., Eichinger, T., Lehr, C.-M., Schaefer, U.F., Windbergs, M., 2014. Characterization and evaluation of a modified PVPA barrier in comparison to Caco-2 cell monolayers for combined dissolution and permeation testing. J. Control. Release 175, 79–86.
Gao, P., Morozowich, W., 2006. Development of supersaturatable self-emulsifying drug delivery system formulations for improving the oral absorption of poorly soluble drugs. Expert opinion on drug delivery 3 (1), 97–110.
Garg, S., Heuck, G., Ip, S., Ramsay, E., 2016. Microfluidics: a transformation tool for nanomedicine development and production. J. Drug Target. 24 (9), 821–835.
Geboers, S., Stappaerts, J., Mols, R., Snoeys, J., Tack, J., Annaert, P., Augustijns, P., 2016. The effect of food on the intraluminal behavior of abiraterone acetate in man. J. Pharm. Sci. 105 (9), 2974–2981.
Georgaka, D., Butler, J., Kesisoglou, F., Reppas, C., Vertzoni, M., 2017. Evaluation of dissolution in the lower intestine and its impact on the absorption process of high dose low solubility drugs. Mol. Pharm. 14 (12), 4181–4191.
Ginski, M., 1999. Prediction of dissolution–absorption relationships from a dissolution/Caco-2 system. Int. J. Pharm. 177 (1), 117–125.
Goole, J., Amighi, K., 2016. 3D printing of pharmaceuticals: a new tool for designing customized drug delivery systems. Int. J. Pharm. 499, 376–394.
Griesser, J., Hetényi, G., Kadas, H., Demarne, F., Jannin, V., Bernkop-Schnürch, A., 2018. Self-emulsifying peptide drug delivery systems: how to make them highly mucus permeating. Int. J. Pharm. 538 (1–2), 159–166.
Griffin, B.T., Kuentz, M., Vertzoni, M., Kostewicz, E.S., Fei, Y., Faisal, W., Stillhart, C., O'Driscoll, C.M., Reppas, C., Dressman, J.B., 2014. Comparison of in vitro tests at various levels of complexity for the prediction of in vivo performance of lipid-based formulations: case studies with fenofibrate. Eur. J. Pharm. Biopharm. 86 (3), 427–437.

Grzybowska, K., Capaccioli, S., Paluch, M., 2016. Recent developments in the experimental investigations of relaxations in pharmaceuticals by dielectric techniques at ambient and elevated pressure. Adv. Drug Deliv. Rev. 100, 158–182.
Hancock, B.C., Shamblin, S.L., 2001. Molecular mobility of amorphous pharmaceuticals determined using differential scanning calorimetry. Thermochim. Acta 380 (95-10).
Hancock, B.C., Shamblin, S.L., Zografi, G., 1995. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. Pharm. Res. 12 (6), 799–806.
Hate, S.S., Reutzel-Edens, S.M., Taylor, L.S., 2019. Insight into amorphous solid dispersion performance by coupled dissolution and membrane mass transfer measurements. Mol. Pharm. 16 (1), 448–461.
Hauss, D.J., 2007. Oral lipid-based formulations. Adv. Drug Deliv. Rev. 59 (7), 667–676.
Hayeshi, R., Hilgendorf, C., Artursson, P., Augustijns, P., Brodin, B., Dehertogh, P., Fisher, K., Fossati, L., Hovenkamp, E., Korjamo, T., et al., 2008. Comparison of drug transporter gene expression and functionality in Caco-2 cells from 10 different laboratories. Eur. J. Pharm. Sci. 35 (5), 383–396.
Hédoux, A., 2016. Recent developments in the Raman and infrared investigations of amorphous pharmaceuticals and protein formulations: a review. Adv. Drug Deliv. Rev. 100, 133–146.
Henry, B.T., Adler, J., Hibberd, S., Cheema, M.S., Davis, S.S., Rogers, T.G., 1992. Epifluorescence microscopy and image analysis used to measure diffusion coefficients in gel systems. J. Pharm. Pharmacol. 44 (7), 543–549.
Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2015. Gastrointestinal behavior of nano- and microsized fenofibrate: in vivo evaluation in man and in vitro simulation by assessment of the permeation potential. Eur. J. Pharm. Sci. 77, 40–47.
Hens, B., Brouwers, J., Corsetti, M., Augustijns, P., 2016a. Supersaturation and precipitation of posaconazole upon entry in the upper small intestine in humans. J. Pharm. Sci. 105 (9), 2677–2684.
Hens, B., Corsetti, M., Brouwers, J., Augustijns, P., 2016b. Gastrointestinal and systemic monitoring of posaconazole in humans after fasted and fed state administration of a solid dispersion. J. Pharm. Sci. 105 (9), 2904–2912.
Hens, B., Corsetti, M., Spiller, R., Marciani, L., Vanuytsel, T., Tack, J., Talattof, A., Amidon, G.L., Koziolek, M., Weitschies, W., Wilson, C.G., Bennink, R.J., Brouwers, J., Augustijns, P., 2017a. Exploring gastrointestinal variables affecting drug and formulation behavior: methodologies, challenges and opportunities. Int. J. Pharm. 519 (1–2), 79–97.
Hens, B., Pathak, S.M., Mitra, A., Patel, N., Liu, B., Patel, S., Jamei, M., Brouwers, J., Augustijns, P., Turner, D.B., 2017b. In silico modeling approach for the evaluation of gastrointestinal dissolution, supersaturation, and precipitation of posaconazole. Mol. Pharm. 14 (12), 4321–4333.
Holm, R., 2019. Bridging the gaps between academic research and industrial product developments of lipid-based formulations. Adv. Drug Deliv. Rev. https://doi.org/10.1016/j.addr.2019.01.009.
Holmboe, M., Larsson, P., Anwar, J., Bergström, C.A.S., 2016. Partitioning into colloidal structures of fasted state intestinal fluid studied by molecular dynamics simulations. Langmuir 32 (48), 12732–12740.
Irvine, J.D., Takahashi, L., Lockhart, K., Cheong, J., Tolan, J.W., Selick, H.E., Grove, J.R., 1999. MDCK (Madin-Darby canine kidney) cells: a tool for membrane permeability screening. J. Pharm. Sci. 88 (1), 28–33.
Jacobsen, A.C., Brandl, M., Bauer-Brandl, A., 2018. High troughput formulation screening: testing a novel two compartment microtiter plate approach. In: Poster AAPS Annual Meeting, . https://www.eventscribe.net/2018/PharmSci360/fsPopup.asp?efp=UUFSQlZZVFM1OTQ2&PosterID=171333&rnd=0.4734136&mode=posterinfo, Accessed date: 4 February 2019.
Jacobsen, A.C., Elvang, P.A., Bauer-Brandl, A., Brandl, M., 2019. A dynamic in vitro permeation study on solid mono- and diacyl-phospholipid dispersions of celecoxib. Eur. J. Pharm. Sci. 127, 199–207.
Jain, N., Yalkowsky, S.H., 2001. Estimation of the aqueous solubility I: application to organic nonelectrolytes. J. Pharm. Sci. 90 (2), 234–252.
Jaisamut, P., Wiwattanawongsa, K., Graidist, P., Sangsen, Y., Wiwattanapatapee, R., 2018. Enhanced oral bioavailability of curcumin using a supersaturatable self-microemulsifying system incorporating a hydrophilic polymer; in vitro and in vivo investigations. AAPS PharmSciTech 19, 730. https://doi.org/10.1208/s12249-017-0857-3.
Jannin, V., 2018. Complex interplay between solubilization, digestion, supersaturation and absorption of poorly soluble drugs with lipid-based formulations. Current drug delivery 15 (6), 749–751.
Jannin, V., Chevrier, S., Michenaud, M., Dumont, C., Belotti, S., Chavant, Y., Demarne, F., 2015. Development of self emulsifying lipid formulations of BCS class II drugs with low to medium lipophilicity. Int. J. Pharm. 495 (1), 385–392.
Jantratid, E., Dressman, J., 2009. Biorelevant dissolution media simulating the proximal human gastrointestinal tract: an update. Dissolution technology 16 (3), 21–25.
Jantratid, E., Janssen, N., Reppas, C., Dressman, J.B., 2008. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm. Res. 25 (7), 1663–1676.
Jørgensen, S.D.S., Al Sawaf, M., Gräeser, K., Mu, H., Müllertz, A., Rades, T., 2018. The ability of two in vitro lipolysis models reflecting the human and rat gastro-intestinal conditions to predict the in vivo performance of SNEDDS dosing regimens. Eur. J. Pharm. Biopharm. 124, 116–124.
Kalman, S.M., Ussing, H.H., 1955. Active sodium uptake by the toad and its response to the antidiuretic hormone. The journal of general physiology 38 (3), 361–370.
Kataoka, M., Masaoka, Y., Sakuma, S., Yamashita, S., 2006. Effect of food intake on the oral absorption of poorly water-soluble drugs: in vitro assessment of drug dissolution and permeation assay system. J. Pharm. Sci. 95 (9), 2051–2061.
Kataoka, M., Itsubata, S., Masaoka, Y., Sakuma, S., Yamashita, S., 2011. In vitro dissolution/permeation system to predict the oral absorption of poorly water-soluble drugs: effect of food and dose strength on it. Biol. Pharm. Bull. 34 (3), 401–407.
Kataoka, M., Tsuneishi, S., Maeda, Y., Masaoka, Y., Sakuma, S., Yamashita, S., 2014. A new in vitro system for evaluation of passive intestinal drug absorption: establishment of a double artificial membrane permeation assay. Eur. J. Pharm. Biopharm. 88 (3), 840–846.
Kaur, N., Narang, A., Bansal, A.K., 2018. Use of biorelevant dissolution and PBPK modeling to predict oral drug absorption. Eur. J. Pharm. Biopharm. 129, 222–246.
Kawakami, K., 2012. Modification of physicochemical characteristics of active pharmaceutical ingredients and application of supersaturatable dosage forms for improving bioavailability of poorly absorbed drugs. Advanced drug delivery review 64, 480–495.
Kearney, P., Marriott, C., 1987. The effects of mucus glycoproteins on the bioavailability of tetracycline. II. Binding. Int. J. Pharm. 35, 211–217.
Keemink, J., Bergström, C.A.S., 2018. Caco-2 cell conditions enabling studies of drug absorption from digestible lipid-based formulations. Pharm. Res. 35 (4), 74. https://doi.org/10.1007/s11095-017-2327-8.
Keemink, J., Mårtensson, E., Bergström, C.A.S., 2019. Lipolysis-permeation setup for simultaneous study of digestion and absorption in vitro. Mol. Pharm. 16 (3), 921–930.
Keserü, G.M., Makara, G.M., 2009. The influence of lead discovery strategies on the properties of drug candidates. Nat. Rev. Drug Discov. 8 (3), 203–212.
Khadra, I., Zhou, Z., Dunn, C., Wilson, C.G., Halbert, G., 2015. Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs. Eur. J. Pharm. Sci. 67, 65–75.
Khan, J., Rades, T., Boyd, B.J., 2018. Addition of cationic surfactants to lipid-based formulations of poorly water-soluble acidic drugs alters the phase distribution and the solid-state form of the precipitate upon in vitro lipolysis. J. Pharm. Sci. 107 (9), 2420–2427.
Khatri, P., Shah, M.K., Vora, N., 2018. Formulation strategies for solid oral dosage forms using 3D printing technology: a mini-review. Journal of Drug Delivery Science and Technology 46, 148–155.
Klein, S., Stein, J., Dressman, J., 2005. Site-specific delivery of anti-inflammatory drugs in the gastrointestinal tract: an in-vitro release model. J. Pharm. Pharmacol. 57, 709–719.
Knoebel, R.W., Larson, R.A., 2018. Pepsi® or Coke®? Influence of acid on dasatinib absorption. J. Oncol. Pharm. Pract. 24 (2), 156–158.
Knopp, M.M., Wendelboe, J., Holm, R., Rades, T., 2018. Effect of amorphous phase separation and crystallization on the in vitro and in vivo performance of an amorphous solid dispersion. Eur. J. Pharm. Biopharm. 130, 290–295.
Koenigsknecht, M.J., Baker, J.R., Wen, B., Frances, A., Zhang, H., Yu, A., Zhao, T., Tsume, Y., Pai, M.P., Bleske, B.E., et al., 2017. In vivo dissolution and systemic absorption of immediate release ibuprofen in human gastrointestinal tract under fed and fasted conditions. Mol. Pharm. 14 (12), 4295–4304.
Koplin, S., Kumpugdee-Vollrath, M., Bauer-Brandl, A., Brandl, M., 2017. Surfactants enhance recovery of poorly soluble drugs during microdialysis sampling: implications for in vitro dissolution – /permeation-studies. J. Pharm. Biomed. Anal. 145, 586–592.
Korn, C., Balbach, S., 2014. Compound selection for development – is salt formation the ultimate answer? Experiences with an extended concept of the “100mg approach”. Eur. J. Pharm. Sci. 57, 257–263.
Kostewicz, E.S., Aarons, L., Bergstrand, M., Bolger, M.B., Galetin, A., Hatley, O., Jamei, M., Lloyd, R., Pepin, X., Rostami-Hodjegan, A., et al., 2014. PBPK models for the prediction of in vivo performance of oral dosage forms. Eur. J. Pharm. Sci. 57, 300–321.
Kourentas, A., Vertzoni, M., Symillides, M., Goumas, K., Gibbon, R., Butler, J., Reppas, C., 2016a. Effectiveness of supersaturation promoting excipients on albendazole concentrations in upper gastrointestinal lumen of fasted healthy adults. Eur. J. Pharm. Sci. 91, 11–19.
Kourentas, A., Vertzoni, M., Symillides, M., Hens, B., Brouwers, J., Augustijns, P., Reppas, C., 2016b. In vitro evaluation of the impact of gastrointestinal transfer on luminal performance of commercially available products of posaconazole and itraconazole using BioGIT. Int. J. Pharm. 515 (1–2), 352–358.
Kourentas, A., Vertzoni, M., Stavrinoudakis, N., Symillidis, A., Brouwers, J., Augustijns, P., Reppas, C., Symillides, M., 2016c. An in vitro biorelevant gastrointestinal transfer (BioGIT) system for forecasting concentrations in the fasted upper small intestine: design, implementation, and evaluation. Eur. J. Pharm. Sci. 82, 106–114.
Koziolek, M., Grimm, M., Garbacz, G., Kühn, J.P., Weitschies, W., 2014. Intragastric volume changes after intake of a high-caloric, high-fat standard breakfast in healthy human subjects investigated by MRI. Mol. Pharm. 11, 1632–1639.
Krishna, A.K., Flanagan, D.R., 1989. Micellar solubilization of a new antimalarial drug, β-Arteether. J. Pharm. Sci. 78 (7), 574–576.
Kuentz, M., 2014. Analytical technologies for real-time drug dissolution and precipitation testing on a small-scale. J. Pharm. Pharmacol. 67, 143–159.
Kuentz, M., 2018. Drug supersaturation during formulation digestion, including real-time analytical approaches. Advanced drug delivery review (ahead of print). https://doi.org/10.1016/j.addr.2018.11.003.
Kuentz, M., Holm, R., Elder, D., 2016. Methodology of oral formulation selection in the pharmaceutical industry. Eur. J. Pharm. Sci. 87, 136–163.
Kuminek, G., Cao, F., Bahia de Oliveira da Rocha, A., Gonçalves Cardoso, S., Rodríguez-Hornedo, N., 2016. Cocrystals to facilitate delivery of poorly soluble compounds beyond-rule-of-5. Adv. Drug Deliv. Rev. 101, 143–166.
Ladas, S.D., Isaacs, P.E., Murphy, G.M., Sladen, G.E., 1984. Comparison of the effects of medium and long chain triglyceride containing liquid meals on gall bladder and small intestinal function in normal man. Gut 25 (4), 405–411.
Laitinen, R., Löbmann, K., Grohganz, H., Priemel, P., Strachan, C.J., Rades, T., 2017. Supersaturating drug delivery systems: the potential of co-amorphous drug formulations. Int. J. Pharm. 532 (1), 1–12.
Larhed, A.W., Artursson, P., Gråsjö, J., Björk, E., 1997. Diffusion of drugs in native and purified gastrointestinal mucus. J. Pharm. Sci. 86 (6), 660–665.
Larsen, A.T., Sassene, P., Müllertz, A., 2011. In vitro lipolysis models as a tool for the characterization of oral lipid and surfactant based drug delivery systems. Int. J. Pharm. 417 (1–2), 245–255.
Larsen, A.T., Ohlsson, A.G., Polentarutti, B., Barker, R.A., Phillips, A.R., Abu-Rmaileh, R., Dickinson, P.A., Abrahamsson, B., Ostergaard, J., Müllertz, A., 2013. Oral bioavailability of cinnarizine in dogs: relation to SNEDDS droplet size, drug solubility and in vitro precipitation. Eur. J. Pharm. Sci. 48, 339–350.

Larsson, P., Alskär, L.C., Bergström, C.A.S., 2017. Molecular structuring and phase transition of lipid-based formulations upon water dispersion: a coarse-grained molecular dynamics simulation approach. Mol. Pharm. 14 (12), 4145–4153.
Lautenschläger, C., Schmidt, C., Lehr, C.M., Fischer, D., Stallmach, A., 2013. PEG-functionalized microparticles selectively target inflamed mucosa in inflammatory bowel disease. Eur. J. Pharm. Biopharm. 85 (3, Part A), 578–586.
Lawless, M.S., Waldman, M., Fraczkiewicz, R., Clarck, R.D., 2016. Using cheminformatics in drug discovery. In: Nielsch, U., Fuhrmann, U., Jaroch, St. (Eds.), New Approaches to Drug Discovery, pp. 139–168.
Lechanteur, A., das Neves, J., Sarmento, B., 2018. The role of mucus in cell-based models used to screen mucosal drug delivery. Adv. Drug Deliv. Rev. 124, 50–63.
Leeson, P.D., Springthorpe, B., 2007. The influence of drug-like concepts on decision-making in medicinal chemistry. Nat. Rev. Drug Discov. 6 (11), 881–890.
Leeson, P.D., St-Gallay, S.A., 2011. The influence of the 'organizational factor' on compound quality in drug discovery. Nat. Rev. Drug Discov. 10 (10), 749–765.
Li, P., Tan, A., Prestidge, C.A., Nielsen, H.M., Müllertz, A., 2014. Self-nanoemulsifying drug delivery systems for oral insulin delivery: in vitro and in vivo evaluations of enteric coating and drug loading. Int. J. Pharm. 477 (1–2), 390–398.
Li, J., Li, L.B., Nessah, N., Huang, Y., Hidalgo, C., Owen, A., Hidalgo, I.J., 2019. Simultaneous analysis of dissolution and permeation profiles of nanosized and microsized formulations of indomethacin using the in vitro dissolution absorption system 2. J. Pharm. Sci. https://doi.org/10.1016/j.xphs.2019.01.032.
Liang, K., Brambilla, D., Leroux, J.C., 2019. Is 3D printing of pharmaceuticals a disruptor or enabler? Adv. Mater. 31, 1805680.
Lichtenberger, L.M., 1995. The hydrophobic barrier properties of gastrointestinal mucus. Annu. Rev. Physiol. 57, 565–583.
Lindmark, T., Nikkilä, T., Artursson, P., 1995. Mechanisms of absorption enhancement by medium chain fatty acids in intestinal epithelial Caco-2 cell monolayers. J. Pharmacol. Exp. Ther. 275 (2), 958–964.
Liu, W., He, X., Li, Z., Gao, X., Ma, Y., Xun, M., Liu, C., 2013. Development of a bionic system for the simultaneous prediction of the release/absorption characteristics of enteric-coated formulations. Pharm. Res. 30 (2), 596–605.
Liu, H., Taylor, L.S., Edgar, K.J., 2015. The role of polymers in oral bioavailability enhancement; a review. Polymer 77, 399–415.
Lo, Y.Ch., Rensi, St.E., Torng, W., Altman, R.B., 2018. Machine learning in cheminfomatics and drug discovery. Drug Discov. Today 23 (8), 1538–1546.
Löbenberg, R., Krämer, J., Shah, V.P., Amidon, G.L., Dressman, J.B., 2000. Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. Pharm. Res. 17 (4), 439–444.
Lundquist, P., Artursson, P., 2016. Oral absorption of peptides and nanoparticles across the human intestine: opportunities, limitations and studies in human tissues. Advanced drug delivery review 106 (Pt B), 256–276.
Madsen, C.M., Feng, K.I., Leithead, A., Canfield, N., Jørgensen, S.A., Müllertz, A., Rades, T., 2017. Effect of composition of simulated intestinal media on the solubility of poorly soluble compounds investigated by design of experiments. Eur. J. Pharm. Sci. 111, 311–319.
Mahler, G.J., Shuler, M.L., Glahn, R.P., 2009. Characterization of Caco-2 and HT29-MTX cocultures in an in vitro digestion/cell culture model used to predict iron bioavailability. J. Nutr. Biochem. 20 (7), 494–502.
Mahmood, A., Laffleur, F., Leonaviciute, G., Bernkop-Schnürch, A., 2017. Protease-functionalized mucus penetrating microparticles: in-vivo evidence for their potential. Int. J. Pharm. 532 (1), 177–184.
Mäkilä, E., Kivelä, H., Shrestha, N., Correia, A., Kaasalainen, M., Kukk, E., Hirvonen, J., Santos, H.A., Salonen, J., 2016. Influence of surface chemistry on ibuprofen adsorption and confinement in mesoporous silicon microparticles. Langmuir 32 (49), 13020–13029.
Markopoulos, C., Andreas, C.J., Vertzoni, M., Dressman, J., Reppas, C., 2015. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: choosing the appropriate test media. Eur. J. Pharm. Biopharm. 93, 173–182.
Marques, M.R.C., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolution technology 18 (3), 15–28.
Marriott, C., Kellaway, K.W., 1975. The effect of tetracyclines on the viscoelastic properties of bronchial mucus. Biorheology 12 (6), 391–395.
Masaoka, Y., Tanaka, Y., Kataoka, M., Sakuma, S., Yamashita, S., 2006. Site of drug absorption after oral administration: assessment of membrane permeability and luminal concentration of drugs in each segment of the gastrointestinal tract. Eur. J. Pharm. Sci. 29, 240–250.
Mathias, N.R., Xu, Y., Patel, D., Grass, M., Caldwell, B., Jager, C., Mullin, J., Hansen, L., Crison, J., Saari, A., 2013. Assessing the risk of pH-dependent absorption for new molecular entities: a novel in vitro dissolution test, physicochemical analysis, and risk assessment strategy. Mol. Pharm. 10, 4063–4073.
Matsson, P., Doak, B.C., Over, B., Kihlberg, J., 2016. Cell permeability beyond the rule of 5. Adv. Drug Deliv. Rev. 101, 42–61.
Maurin, M.B., Grant, D.J., Stahl, P.H., 2002. The physicochemical background: fundamentals of ionic equilibria. In: Stahl, P.H., Wermuth, C.G. (Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use. Wiley-VCH, Weinheim, pp. 9–17.
Miller, J.M., Beig, A., Krieg, B.J., Carr, R.A., Borchardt, T.B., Amidon, G.E., Amidon, G.L., Dahan, A., 2011. The solubility permeability interplay: mechanistic modeling and predictive application of the impact of micellar solubilization on intestinal permeation. Mol. Pharm. 8 (5), 1848–1856.
Miller, J.M., Beig, A., Carr, R.A., Webster, G.K., Dahan, A., 2012. The solubility−−permeability interplay when using cosolvents for solubilization: revising the way we use solubility-enabling formulations. Mol. Pharm. 9 (3), 581–590.
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., et al., 2014. A standardised static in vitro digestion method suitable for food - an international consensus. Food Funct. 5 (6), 1113–1124.
Mitic, A., Gernaey, K.V., 2016. Process intensification tools in the small-scale pharmaceutical manufacturing of small molecules. Chemical engineering & technology 38 (10), 1699–1712.
Mohsin, K., Long, M.A., Pouton, C.W., 2009. Design of lipid-based formulations for oral administration of poorly water-soluble drugs: precipitation of drug after dispersion of formulations in aqueous solution. J. Pharm. Sci. 98 (10), 3582–3595.
Motz, S.A., Schaefer, U.F., Balbach, S., Eichinger, T., Lehr, C.-M., 2007. Permeability assessment for solid oral drug formulations based on Caco-2 monolayer in combination with a flow through dissolution cell. Eur. J. Pharm. Biopharm. 66 (2), 286–295.
Mu, H., Holm, R., Müllertz, A., 2013. Lipid-based formulations for oral administration of poorly water-soluble drugs. Int. J. Pharm. 453 (1), 215–224.
Müller, C., Leithner, K., Hauptstein, S., Hintzen, F., Salvenmoser, W., Bernkop-Schnürch, A., 2013. Preparation and characterization of mucus-penetrating papain/poly(acrylic acid) nanoparticles for oral drug delivery applications. J. Nanopart. Res. 15 (1), 1.
Müllertz, A., Reppas, C., Psachoulias, D., Vertzoni, M., Fatouros, D.G., 2015. Structural features of colloidal species in the human fasted upper small intestine. J. Pharm. Pharmacol. 67 (4), 486–492.
Mura, P., Valleri, M., Fabianelli, E., Maestrelli, F., Cirri, M., 2019. Characterization and evaluation of different mesoporous silica kinds as carriers for the development of effective oral dosage forms of glibenclamide. Int. J. Pharm. 563, 43–52.
Murakami, T., 2016. A minireview: usefulness of transporter-targeted prodrugs in enhancing membrane permeability. J. Pharm. Sci. 105 (9), 2515–2526.
Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., et al., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384 (9945), 766–781.
N'Goma, J.-C.B., Amara, S., Dridi, K., Jannin, V., Carrière, F., 2012. Understanding the lipid-digestion processes in the GI tract before designing lipid-based drug-delivery systems. Ther. Deliv. 3 (1), 105–124.
Nie, H., Byrn, S.R., Zhou, Q., 2017. Stability of pharmaceutical salts in solid oral dosage forms. Drug Dev. Ind. Pharm. 43 (8), 1215–1228.
Norman, J., Madurawe, R.D., Moore, C.M.V., Khan, M.A., Khairuzzaman, A., 2017. A new chapter in pharmaceutical manufacturing: 3D-printed drug products. Advanced drug Delivery Review 108, 39–50.
Nunes, R., Silva, C., Chaves, L., 2016. 4.2 - Tissue-based in vitro and ex vivo models for intestinal permeability studies. In: Sarmento, B. (Ed.), Concepts and Models for Drug Permeability Studies. Woodhead Publishing, pp. 203–236.
Occhipinti, P., Griffiths, P.C., 2008. Quantifying diffusion in mucosal systems by pulsed-gradient spin-echo NMR. Adv. Drug Deliv. Rev. 60 (15), 1570–1582.
O'Connor, K.M., Corrigan, O.I., 2001. Preparation and characterisation of a range of diclofenac salts. Int. J. Pharm. 226 (1–2), 163–179.
Ong, J.T.H., Manoukian, E., 1988. Micellar solubilization of timobesone acetate in aqueous and aqueous propylene glycol solutions of nonionic surfactants. Pharm. Res. 05 (11), 704–708.
Palm, K., Stenberg, P., Luthman, K., Artursson, P., 1997. Polar molecular surface properties predict the intestinal absorption of drugs in humans. Pharm. Res. 14 (5), 568–571.
Palmelund, H., Madsen, C.M., Plum, J., Müllertz, A., Rades, T., 2016. Studying the propensity of compounds to supersaturate: a practical and broadly applicable approach. J. Pharm. Sci. 105, 3021–3029.
Park, S.-H., Choi, H.-K., 2006. The effects of surfactants on the dissolution profiles of poorly water-soluble acidic drugs. Int. J. Pharm. 321 (1–2), 35–41.
Pathak, S.M., Ruff, A., Kostewicz, E.S., Patel, N., Turner, D.B., Jamei, M., 2017. Model-based analysis of biopharmaceutic experiments to improve mechanistic oral absorption modeling: an integrated in vitro in vivo extrapolation perspective using ketoconazole as a model drug. Mol. Pharm. 14, 4305–4320.
Paulekuhn, G.S., Dressman, J.B., Saal, C., 2007. Trends in active pharmaceutical ingredient salt selection based on analysis of the orange book database. J. Med. Chem. 50 (26), 6665–6672.
Pearce, S.C., Coia, H.G., Karl, J.P., Pantoja-Feliciano, I.G., Zachos, N., Racicot, K., 2018. Intestinal in vitro and ex vivo models to study host-microbiome interactions and acute stressors. Front. Physiol. 9 (1584).
Peppas, N.A., Thomas, J.B., McGinity, J., 2009. Molecular aspects of mucoadhesive carrier development for drug delivery and improved absorption. Journal of biomaterials science 20, 1–20.
Pereira de Sousa, I., Steiner, C., Schmutzler, M., Wilcox, M.D., Veldhuis, G.J., Pearson, J.P., Huck, C.W., Salvenmoser, W., Bernkop-Schnürch, A., 2015. Mucus permeating carriers: formulation and characterization of highly densely charged nanoparticles. Eur. J. Pharm. Biopharm. 97 (Pt A), 273–279.
Pereira, C., Costa, J., Sarmento, B., Araújo, F., 2016. 3.3 - Cell-based in vitro models for intestinal permeability studies. In: Sarmento, B. (Ed.), Concepts and Models for Drug Permeability Studies. Woodhead Publishing, pp. 57–81.
Pereira-Caro, G., Mateos, R., Saha, S., Madrona, A., Espartero, J.L., Bravo, L., Kroon, P.A., 2010. Transepithelial transport and metabolism of new lipophilic ether derivatives of hydroxytyrosol by enterocyte-like Caco-2/TC7 cells. J. Agric. Food Chem. 58 (21), 11501–11509.
Phillips, D.J., Pygall, S.R., Cooper, V.B., Mann, J.C., 2012. Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems. J. Pharm. Pharmacol. 64 (11), 1549–1559.
Plum, J., Madsen, C.M., Teleki, A., Bevernage, J., da Costa, M.C., Karlsson, E.M., Carlert, S., Holm, R., Müller, T., Matthews, W., et al., 2017. Investigation of the intra- and interlaboratory reproducibility of a small scale standardized supersaturation and precipitation method. Mol. Pharm. 14, 4161–4169.
Porter, C.J.H., Trevaskis, N.L., Charman, W.N., 2007. Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. Nat. Rev. Drug Discov. 6 (3), 231–248.
Pouton, C.W., 2006. Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. Eur. J. Pharm. Sci. 29, 278–287.
Pouton, C.W., Porter, C.J.H., 2008. Formulation of lipid-based delivery systems for oral

administration: materials, methods and strategies. Adv. Drug Deliv. Rev. 60 (6), 625–637.
Psachoulias, D., Vertzoni, M., Goumas, K., Kalioras, V., Beato, S., Butler, J., Reppas, C., 2011. Precipitation in and supersaturation of contents of the upper small intestine after administration of two weak bases to fasted adults. Pharm. Res. 28 (12), 3145–3158.
Quan, G., Niu, B., Singh, V., Zhou, Y., Wu, C., Pan, X., Wu, C., 2017. Supersaturable solid self-microemulsifying drug delivery system: precipitation inhibition and bioavailability enhancement. Int. J. Nanomedicine 12, 8801–8811.
Raina, S.A., Zhang, G.G.Z., Alonzo, D.E., Wu, J., Zhu, D., Catron, N.D., Gao, Y., Taylor, L.S., 2015. Impact of solubilizing additives on supersaturation and membrane transport of drug. Pharm. Res. 32 (10), 3350–3364.
Ramsundar, B., Liu, B., Wu, Z., Verras, A., Tudor, M., Sheridan, R.P., Pande, V., 2017. Is multitask deep learning practical for pharma? J. Chem. Inf. Model. 57, 2068–2076.
Rantanen and Khinast, 2015. The future of pharmaceutical manufacturing sciences. J. Pharm. Sci. 104, 3612–3638.
Raumer, V.M., Dannappel, J., Hilfiker, R., 2006. Polymorphism, salts, and crystallization: the relevance of solid state development. Chemistry Today 24, 41–44.
Roeselers, G., Roeselers, G., Ponomarenko, M., Lukovac, S., Wortelboer, H.M., 2013. Ex vivo systems to study host–microbiota interactions in the gastrointestinal tract. Best Pract. Res. Clin. Gastroenterol. 27 (1), 101–113.
Rojas, J., Buckner, I., Kumar, V., 2012. Co-processed excipients with enhanced direct compression functionality for improved tableting performance. Drug development and industrial pharmacy38(10), 1159–1170.
Rosen, M.J., 2004. Surfactants and Interfacial Phenomena, 3rd ed. John Wiley & Sons, Inc., New Jersey.
Rosenberger, J., Butler, J., Dressman, J., 2018. A refined developability classification system. J. Pharm. Sci. 107, 2020–2032.
Rossi Sebastiano, M., Doak, B.C., Backlund, M., Poongavanam, V., Over, B., Ermondi, G., Caron, G., Matsson, P., Kihlberg, J., 2018. Impact of dynamically exposed polarity on permeability and solubility of chameleonic drugs beyond the rule of 5. J. Med. Chem. 61 (9), 4189–4202.
Rozehnal, V., Nakai, D., Hoepner, U., Fischer, T., Kamiyama, E., Takahashi, M., Yasuda, S., Mueller, J., 2012. Human small intestinal and colonic tissue mounted in the Ussing chamber as a tool for characterizing the intestinal absorption of drugs. Eur. J. Pharm. Sci. 46 (5), 367–373.
Rubbens, J., Brouwers, J., Tack, J., Augustijns, P., 2016. Gastrointestinal dissolution, supersaturation and precipitation of the weak base indinavir in healthy volunteers. Eur. J. Pharm. Biopharm. 109, 122–129.
Sams, L., Amara, S., Mansuelle, P., Puppo, R., Lebrun, R., Paume, J., Giallo, J., Carrière, F., 2018. Characterization of pepsin from rabbit gastric extract, its action on b-casein and the effects of lipids on proteolysis. Food Funct. 9, 5975–5988.
Sarmento, B., Andrade, F., da Silva, S.B., Rodrigues, F., das Neves, J., Ferreira, D., 2012. Cell-based in vitro models for predicting drug permeability. Expert Opin. Drug Metab. Toxicol. 8 (5), 607–621.
Sarode, A.L., Sandhu, H., Shah, N., Malick, W., Zia, H., 2013. Hot melt extrusion (HME) for amorphous solid dispersions: predictive tools for processing and impact of drug–polymer interactions on supersaturation. Eur. J. Pharm. Sci. 48 (3), 371–384.
Sassene, P.J., Knopp, M.M., Hesselkilde, J.Z., Koradia, V., Larsen, A., Rades, T., Müllertz, A., 2010. Precipitation of a poorly soluble model drug during in vitro lipolysis: characterization and dissolution of the precipitate. J. Pharm. Sci. 99 (12), 4982–4991.
Sassene, P., Kleberg, K., Williams, H.D., Bakala-N'Goma, J.-C., Carrière, F., Calderone, M., Jannin, V., Igonin, A., Partheil, A., Marchaud, D., et al., 2014. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 6: effects of varying pancreatin and calcium levels. AAPS J. 16 (6), 1344–1357.
Sassene, P.J., Mosgaard, M.D., Löbmann, K., Mu, H., Larsen, F.H., Rades, T., Müllertz, A., 2015. Elucidating the molecular interactions occurring during drug precipitation of weak bases from lipid-based formulations: a case study with cinnarizine and a long chain self-nanoemulsifying drug delivery system. Mol. Pharm. 12 (11), 4067–4076.
Schiller, C., Frölich, C.P., Giessman, T., Siegmund, W., Mönnikes, H., Hosten, N., Weitschies, W., 2005. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. Aliment. Pharmacol. Ther. 22, 971–979.
Serajuddin, A.T.M., 2002. A Systematic Approach to the Identification of Chemical and Physical Forms, Presented at AAPS Workshop on Chemical and Physical for Selection of Drug Candidates, American Association of Pharmaceutical Scientists, Arlington, VA, (April 25–26, 2002).
Serajuddin, A.T.M., 2007. Salt formation to improve drug solubility. Adv. Drug Deliv. Rev. 59 (7), 603–616.
Serajuddin, A.T.M., Jarowski, C.I., 1993. Influence of pH on release of phenytoin sodium from slow-release dosage forms. J. Pharm. Sci. 82, 306–310.
Shalaev, E., Wu, K., Shamblin, S., Krzyzaniak, J.F., Descamps, M., 2016. Crystalline mesophases: structure, mobility, and pharmaceutical properties. Adv. Drug Deliv. Rev. 100, 194–211.
Shamshina, J.L., Barber, P.S., Rogers, R.D., 2013. Ionic liquids in drug delivery. Expert Opinion on Drug Delivery 10 (10), 1367–1381.
Shrestha, N., Bouttefeux, O., Vanvarenberg, K., Lundquist, P., Cunarro, J., Tovar, S., Khodus, G., Andersson, E., Keita, Å.V., Gonzalez Dieguez, C., et al., 2018. The stimulation of GLP-1 secretion and delivery of GLP-1 agonists via nanostructured lipid carriers. Nanoscale 10 (2), 603–613.
Sibik, J., Zeitler, J.A., 2016. Direct measurement of molecular mobility and crystallisation of amorphous pharmaceuticals using terahertz spectroscopy. Adv. Drug Deliv. Rev. 100, 147–157.
Sigurdsson, H., Kirch, J., Lehr, C.M., 2013. Mucus as barrer to lipophilic drugs. Int. J. Pharm. 453, 56–64.
Simon, L.L., Pataki, H., Marosi, G., Meemken, F., Hungerbühler, K., Baiker, A., Tummala, S., Glennon, B., Kuentz, M., Steele, G., et al., 2015. Assessment of recent process analytical technology (PAT) trends: a multiauthor review. Org. Process Res. Dev. 19 (1), 3–62.
Singh, A., Van den Mooter, G., 2016. Spray drying formulation of amorphous solid dispersions. Adv. Drug Deliv. Rev. 100, 27–50.
Siqueira, S.D., Müllertz, A., Gräeser, K., Kasten, G., Mu, H., Rades, T., 2017. Influence of drug load and physical form of cinnarizine in new SNEDDS dosing regimens: in vivo and in vitro evaluations. AAPS J. 19 (2), 587–594.
Sironi, D., Christensen, M., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2017a. Evaluation of a dynamic dissolution/permeation model: mutual influence of dissolution and barrier-flux under non-steady state conditions. Int. J. Pharm. 522 (1–2), 50–57.
Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2017b. Dynamic dissolution-/permeation-testing of nano- and microparticle formulations of fenofibrate. Eur. J. Pharm. Sci. 96, 20–27.
Sironi, D., Rosenberg, J., Bauer-Brandl, A., Brandl, M., 2018. PermeaLoop™, a novel in vitro tool for small-scale drug-dissolution/permeation studies. J. Pharm. Biomed. Anal. 156, 247–251.
Six, K., Daems, T., de Hoon, J., van Hecken, A., Depre, M., Bouche, M.-P., Prinsen, P., Verreck, G., Peeters, J., Brewster, M.E., van den Mooter, G., 2005. Clinical study of solid dispersions of itraconazole prepared by hot-stage extrusion. Eur. J. Pharm. Sci. 24 (2–3), 179–186.
Sjöberg, Å., Lutz, M., Tannergren, C., Wingolf, C., Borde, A., Ungell, A.L., 2013. Comprehensive study on regional human intestinal permeability and prediction of fraction absorbed of drugs using the Ussing chamber technique. Eur. J. Pharm. Sci. 48 (1), 166–180.
Smith, J.S., Roitberg, A.E., Isayev, O., 2018. Transforming computational drug discovery with machine learning and AI. ACS Med. Chem. Lett. 9 (11), 1065–1069.
Stahl, P.H., Nakano, M., 2002. Pharmaceutical aspects of the drug salt form. In: Stahl, P.H., Wermuth, C.G. (Eds.), Handbook of Pharmaceutical Salts: Properties, Selection, and Use. Wiley-VCH, Weinheim, pp. 9–17.
Stahl, P.H., Wermuth, C.G., 2008. Handbook of Pharmaceutical Salts Properties, Selection, and Use. John Wiley & Sons.
Stappaerts, J., Geboers, S., Snoeys, J., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2015. Rapid conversion of the ester prodrug abiraterone acetate results in intestinal supersaturation and enhanced absorption of abiraterone: in vitro, rat in situ and human in vivo studies. Eur. J. Pharm. Biopharm. 90, 1–7.
Stephenson, G.A., Arurub, A., Woods, T.A., 2011. Physical stability of salts of weak bases in the solid-state. J. Pharm. Sci. 100, 1607–1617.
Stewart, A.M., Grass, M.E., Brodeur, T.J., Goodwin, A.K., Morgen, M.M., Friesen, D.T., Vodak, D.T., 2017a. Impact of drug-rich colloids of itraconazole and HPMCAS on membrane flux in vitro and oral bioavailability in rats. Mol. Pharm. 14 (7), 2437–2449.
Stewart, A.M., Grass, M.E., Mudie, D.M., Morgen, M.M., Friesen, D.T., Vodak, D.T., 2017b. Development of a biorelevant, material-sparing membrane flux test for rapid screening of bioavailability-enhancing drug product formulations. Mol. Pharm. 14 (6), 2032–2046.
Stillhart, C., Imanidis, G., Kuentz, M., 2013. Insights into drug precipitation kinetics during in vitro digestion of a lipid-based drug delivery system using in-line Raman spectroscopy and mathematical modeling. Pharm. Res. 30, 3114–3130.
Stillhart, C., Imanidis, G., Griffin, B.T., Kuentz, M., 2014. Biopharmaceutical modeling of drug supersaturation during lipid-based formulation digestion considering an absorption sink. Pharm. Res. 31, 3426–3444.
Stoyanova, K., Vinarov, Z., Tcholakova, S., 2016. Improving ibuprofen solubility by surfactant-facilitated self-assembly into mixed micelles. Journal of drug delivery science and technology 36, 208–215.
Sun, D.D., Wen, H., Taylor, L.S., 2016. Non-sink dissolution conditions for predicting product quality and in vivo performance of supersaturating drug delivery systems. J. Pharm. Sci. 105 (9), 2477–2488.
Sun, M., Li, B., Li, Y., Liu, Y., Liu, Q., Jiang, H., He, Z., Zhao, Y., Sun, J., 2017. Experimental observations and dissipative particle dynamic simulations on microstructures of pH-sensitive polymer containing amorphous solid dispersions. Int. J. Pharm. 517 (1–2), 185–195.
Suys, E.J.A., Warren, D.B., Porter, C.J.H., Benameur, H., Pouton, C.W., Chalmers, D.K., 2017. Computational models of the intestinal environment. 3. The impact of cholesterol content and pH on mixed micelle colloids. Mol. Pharm. 14 (11), 3684–3697.
Suys, E.J.A., Chalmers, D.K., Pouton, C.W., Porter, C.J.H., 2018. Polymeric precipitation inhibitors promote fenofibrate supersaturation and enhance drug absorption from a type IV lipid-based formulation. Mol. Pharm. 15 (6), 2355–2371.
Szilárd, P., Abraham, M.J., Kutzner, C., Hess, B., Lindahl, E., 2015. Tackling Exascale software challenges in molecular dynamics simulations with GROMACS. Proc. EASC 2014 (8759), 3–27.
Tavelin, S., Taipalensuu, J., Hallböök, F., Vellonen, K.-S., Moore, V., Artursson, P., 2003. An improved cell culture model based on 2/4/A1 cell monolayers for studies of intestinal drug transport: characterization of transport routes. Pharm. Res. 20 (3), 373–381.
Taylor, L.S., Zhang, G.G.Z., 2016. Physical chemistry of supersaturated solutions and implications for oral absorption. Adv. Drug Deliv. Rev. 101, 122–142.
Terao, T., Matsuda, K., Shouji, H., 2001. Improvement in site-specific intestinal absorption of furosemide by Eudragit L100-5. J. Pharm. Pharmacol. 53, 433–440.
Thakral, S., Terban, M.W., Thakral, N.K., Suryanarayanan, R., 2016. Recent advances in the characterization of amorphous pharmaceuticals by X-ray diffractometry. Adv. Drug Deliv. Rev. 100, 183–193.
Tho, I., Liepold, B., Rosenberg, J., Maegerlein, M., Brandl, M., Fricker, G., 2010. Formation of nano/micro-dispersions with improved dissolution properties upon dispersion of ritonavir melt extrudate in aqueous media. Eur. J. Pharm. Sci. 40 (1), 25–32.
Tibbitt, M.W., Dahlman, J.E., Langer, R., 2016. Emerging frontiers in drug delivery. J. Am. Chem. Soc. 138 (3), 704–717.
Tran, T., Siqueira, S.D.V.S., Amenitsch, H., Müllertz, A., Rades, T., 2017a. In vitro and in vivo performance of monoacyl phospholipid-based self-emulsifying drug delivery systems. J. Control. Release 255, 45–53.
Tran, T., Siqueira, S.D.V.S., Amenitsch, H., Rades, T., Müllertz, A., 2017b. Monoacyl phosphatidylcholine inhibits the formation of lipid multilamellar structures during in vitro lipolysis of self-emulsifying drug delivery systems. Eur. J. Pharm. Sci. 108,

62–70.
Tran, T., Chakraborty, A., Xi., X., Bohets, H., Cornett, Cl., Tsinman, K., Rades, Th., Müllertz, A., 2018. Using potentiometric free drug sensors to determine the free concentration of ionizable drugs in colloidal systems. J. Pharm. Sci. 107 (1), 103–112.
Trevaskis, N.L., Charman, W.N., Porter, C.J.H., 2008. Lipid-based delivery systems and intestinal lymphatic drug transport: a mechanistic update. Adv. Drug Deliv. Rev. 60 (6), 702–716.
Tsinman, K., Tsinman, O., Lingamaneni, R., Zhu, S., Riebesehl, B., Grandeury, A., Juhnke, M., Van Eerdenbrugh, B., 2018. Ranking Itraconazole formulations based on the flux through artificial lipophilic membrane. Pharm. Res. 35 (8). https://doi.org/10.1007/s11095-018-2440-3.
Turco, L., Catone, T., Caloni, F., Di Consiglio, E., Testai, E., Stammati, A., 2011. Caco-2/TC7 cell line characterization for intestinal absorption: how reliable is this in vitro model for the prediction of the oral dose fraction absorbed in human? Toxicol. in Vitro 25 (1), 13–20.
Ubbink, J., 2016. Structural and thermodynamic aspects of plasticization and anti-plasticization in glassy encapsulation and biostabilization matrices. Adv. Drug Deliv. Rev. 100, 10–26.
Ullah, I., Baloch, M.K., Ullah, I., Mustaqeem, M., 2014. Enhancement in aqueous solubility of mefenamic acid using micellar solutions of various surfactants. J. Solut. Chem. 43 (8), 1360–1373.
van den Abeele, J., Brouwers, J., Mattheus, R., Tack, J., Augustijns, P., 2016. Gastrointestinal behavior of weakly acidic BCS class II drugs in man—case study of diclofenac potassium. J. Pharm. Sci. 105 (2), 687–696.
van den Abeele, J., Brouwers, J., Tack, J., Augustijns, P., 2017a. Exploring the link between gastric motility and intragastric drug distribution in man. Eur. J. Pharm. Biopharm. 112, 75–84.
van den Abeele, J., Schilderink, R., Schneider, F., Mols, R., Minekus, M., Weitschies, W., Brouwers, J., Tack, J., Augustijns, P., 2017b. Gastrointestinal and systemic disposition of diclofenac under fasted and fed state conditions supporting the evaluation of in vitro predictive tools. Mol. Pharm. 14 (12), 4220–4232.
van den Abeele, J., Rayyan, M., Hoffman, I., Van de Vijver, E., Zhu, W., Augustijns, P., 2018. Gastric fluid composition in a paediatric population: age-dependent changes relevant for gastrointestinal drug disposition. Eur. J. Pharm. Sci. 123, 301–311.
van den Mooter, G., 2012. The use of amorphous solid dispersions: a formulation strategy to overcome poor solubility and dissolution rate. Drug Discov. Today Technol. e79–e85.
Vasconcelos, T., Marques, S., das Neves, J., Sarmento, B., 2016. Amorphous solid dispersions: rational selection of a manufacturing process. Adv. Drug Deliv. Rev. 100, 85–101.
Veber, D.F., Johnson, S.R., Cheng, H.-Y., Smith, B.R., Ward, K.W., Kopple, K.D., 2002. Molecular properties that influence the oral bioavailability of drug candidates. J. Med. Chem. 45 (12), 2615–2623.
Vertzoni, M., Dressman, J., Butler, J., Hempenstall, J., Reppas, C., 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. Eur. J. Pharm. Biopharm. 60 (3), 413–417.
Vertzoni, M., Markopoulos, C., Symillides, M., Goumas, C., Imanidis, G., Reppas, C., 2012. Luminal lipid phases after administration of a triglyceride solution of danazol in the fed state and their contribution to the flux of danazol across Caco-2 cell monolayers. Mol. Pharm. 9 (5), 1189–1198.
Vieth, M., Sutherland, J.J., 2006. Dependence of molecular properties on proteomic family for marketed oral drugs. J. Med. Chem. 49 (12), 3451–3453.
Vinarov, Z., Dobreva, P., Tcholakova, S., 2018a. Effect of surfactant molecular structure on progesterone solubilization. Journal of drug delivery science and technology 43, 44–49.
Vinarov, Z., Gancheva, G., Katev, V., Tcholakova, S.S., 2018b. Albendazole solution formulation via vesicle-to-micelle transition of phospholipid-surfactant aggregates. Drug Dev. Ind. Pharm. 44 (7), 1130–1138.
Vinarov, Z., Katev, V., Burdzhiev, N., Tcholakova, S., Denkov, N., 2018c. Effect of surfactant-bile interactions on the solubility of hydrophobic drugs in biorelevant dissolution media. Mol. Pharm. https://doi.org/10.1021/acs.molpharmaceut.8b00884.
Vinarov, Z., Katev, V., Radeva, D., Tcholakova, S., Denkov, N.D., 2018d. Micellar solubilization of poorly water-soluble drugs: effect of surfactant and solubilizate molecular structure. Drug Dev. Ind. Pharm. 44 (4), 677–686.
Vithani, K., Hawley, A., Jannin, V., Pouton, C., Boyd, B.J., 2017. Inclusion of digestible surfactants in solid SMEDDS formulation removes lag time and influences the formation of structured particles during digestion. AAPS J. 19 (3), 754–764.
Vithani, K., Goyanes, A., Jannin, V., Gaisford, S., Boyd, B.J., 2019. An overview of 3D printing technologies for soft materials and potential opportunities for lipid-based systems. Pharm. Res. 36 (4). https://doi.org/10.1007/s11095-018-2531-1.
Vo, C.L.N., Park, Ch., Lee, B.J., 2013. Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. European journal of pharmaceutics biopharmaceutics 85, 799–813.
Walravens, J., Brouwers, J., Spriet, I., Tack, J., Annaert, P., Augustijns, P., 2011. Effect of pH and comedication on gastrointestinal absorption of posaconazole: monitoring of intraluminal and plasma drug concentrations. Clin. Pharmacokinet. 50 (11), 725–734.
Wang, W., Jiang, J., Ballard, C.E., Wang, B., 1999. Prodrug approaches to the improved delivery of peptide drugs. Curr. Pharm. Des. 5 (4), 265–287.
Warren, D.B., Benameur, H., Porter, C.J.H., Pouton, C.W., 2010. Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: a mechanistic basis for utility. J. Drug Target. 18 (10), 704–731.
Warren, D.B., Bergström, C.A.S., Benameur, H., Porter, C.J.H., Pouton, C.W., 2013. Evaluation of the structural determinants of polymeric precipitation inhibitors using solvent shift methods and principle component analysis. Mol. Pharm. 10 (8), 2823–2848.
Warren, D.B., Benameur, H., Pouton, C.W., Chalmers, D.K., 2019. Location of solvated probe molecules within non-ionic surfactant micelles using molecular dynamics. J. Pharm. Sci. 108 (1), 205–213.
Wassvik, C.M., Holmén, A.G., Draheim, R., Artursson, P., Bergström, C.A.S., 2008. Molecular characteristics for solid-state limited solubility. J. Med. Chem. 51 (10), 3035–3039.
Westerhout, J., van de Steeg, E., Grossouw, D., Zeijdner, E.E., Krul, C.A.M., Verwei, M., Wortelboeret, H.M., 2014. A new approach to predict human intestinal absorption using porcine intestinal tissue and biorelevant matrices. Eur. J. Pharm. Sci. 63, 167–177.
Westerhout, J., Wortelboer, H., Verhoeckx, K., 2015. Ussing chamber. In: Verhoeckx, K. (Ed.), The Impact of Food Bioactives on Health: In Vitro and Ex Vivo Models, Cham (CH), pp. 263–273.
Wikman-Larhed, A., Artursson, P., 1995. Co-cultures of human intestinal goblet (HT29-H) and absorptive (Caco-2) cells for studies of drug and peptide absorption. European journal of pharmaceutical sciences 3 (3), 171–183.
Williams, H.D., Anby, M.U., Sassene, P., Kleberg, K., Bakala-N'Goma, J.-C., Calderone, M., Jannin, V., Igonin, A., Partheil, A., Marchaud, D., et al., 2012a. Toward the establishment of standardized in vitro tests for lipid-based formulations. 2. The effect of bile salt concentration and drug loading on the performance of type I, II, IIIA, IIIB, and IV formulations during in vitro digestion. Mol. Pharm. 9 (11), 3286–3300.
Williams, H.D., Sassene, P., Kleberg, K., Bakala-N'Goma, J.-C., Calderone, M., Jannin, V., Igonin, A., Partheil, A., Marchaud, D., Jule, E., et al., 2012b. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 1: method parameterization and comparison of in vitro digestion profiles across a range of representative formulations. J. Pharm. Sci. 101 (9), 3360–3380.
Williams, H.D., Sassene, P., Kleberg, K., Calderone, M., Igonin, A., Jule, E., Vertommen, J., Blundell, R., Benameur, H., Müllertz, A., et al., 2013. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 3: understanding supersaturation versus precipitation potential during the in vitro digestion of type I, II, IIIA, IIIB and IV lipid-based formulations. Pharm. Res. 30 (12), 3059–3076.
Williams, H.W., Sahbaz, Y., Ford, L., Nguyen, T.H., Scammells, P.J., Porter, C.J.H., 2014a. Ionic liquids provide unique opportunities for oral drug delivery: structure optimization and in vivo evidence of utility. Chem. Commun. 50, 1688–1690.
Williams, H.D., Sassene, P., Kleberg, K., Calderone, M., Igonin, A., Jule, E., Vertommen, J., Blundell, R., Benameur, H., Müllertz, A., et al., 2014b. Toward the establishment of standardized in vitro tests for lipid-based formulations, part 4: proposing a new lipid formulation performance classification system. J. Pharm. Sci. 103 (8), 2441–2455.
Wuyts, B., Riethorst, D., Brouwers, J., Tack, J., Annaert, P., Augustijns, P., 2015. Evaluation of fasted and fed state simulated and human intestinal fluids as solvent system in the Ussing chambers model to explore food effects on intestinal permeability. Int. J. Pharm. 478 (2), 736–744.
Xiang, T.-X., Anderson, B.D., 2004. A molecular dynamics simulation of reactant mobility in an amorphous formulation of a peptide in poly(vinylpyrrolidone). J. Pharm. Sci. 93 (4), 855–876.
Xiang, T.-X., Anderson, B.D., 2005. Distribution and effect of water content on molecular mobility in poly(vinylpyrrolidone) glasses: a molecular dynamics simulation. Pharm. Res. 22 (8), 1205–1214.
Xiang, T.-X., Anderson, B.D., 2013a. Molecular dynamics simulation of amorphous indomethacin. Mol. Pharm. 10 (1), 102–114.
Xiang, T.-X., Anderson, B.D., 2013b. Molecular dynamics simulation of amorphous indomethacin-poly(vinylpyrrolidone) glasses: solubility and hydrogen bonding interactions. J. Pharm. Sci. 102 (3), 876–891.
Xiang, T.-X., Anderson, B.D., 2014. Molecular dynamics simulation of amorphous hydroxypropyl-methylcellulose acetate succinate (HPMCAS): polymer model development, water distribution, and plasticization. Mol. Pharm. 11 (7), 2400–2411.
Xiang, T.-X., Anderson, B.D., 2019. Effects of molecular interactions on miscibility and mobility of ibuprofen in amorphous solid dispersions with various polymers. J. Pharm. Sci. 108 (1), 178–186.
Yeap, Y.Y., Trevaskis, N.L., Porter, C.J.H., 2013. Lipid absorption triggers drug supersaturation at the intestinal unstirred water layer and promotes drug absorption from mixed micelles. Pharm. Res. 30 (12), 3045–3058.
Yu, L., 2001. Amorphous pharmaceutical solids: preparation, characterization and stabilization. Adv. Drug Deliv. Rev. 48 (1), 27–42.
Yu, H., Xia, D., Zhu, Q., Chen, D., Gan, Y., 2013. Supersaturated polymeric micelles for oral cyclosporine A delivery. European Journal of Pharmaceutics Biopharmaceutics 85, 1325–1336.
Yuan, X., Xiang, T.-X., Anderson, B.D., Munson, E.J., 2015. Hydrogen bonding interactions in amorphous indomethacin and its amorphous solid dispersions with poly(vinylpyrrolidone) and poly(vinylpyrrolidone-co-vinyl acetate) studied using (13)C solid-state NMR. Mol. Pharm. 12 (12), 4518–4528.
Zaki, N.M., Artursson, P., Bergström, C.A.S., 2010. A modified physiological BCS for prediction of intestinal absorption in drug discovery. Mol. Pharm. 7 (5), 1478–1487.
Zhang, Y., Wang, J., Bai, X., Jiang, T., Zhang, Q., Wang, S., 2012. Mesoporous silica nanoparticles for increasing the oral bioavailability and permeation of poorly water soluble drugs. Mol. Pharm. 9 (3), 505–513.