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In vivo methods for drug absorption – Comparative physiologies, model selection, correlations with *in vitro* methods (IVIVC), and applications for formulation/API/excipient characterization including food effects

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ABSTRACT

This review summarizes the current knowledge on anatomy and physiology of the human gastrointestinal tract in comparison with that of common laboratory animals (dog, pig, rat and mouse) with emphasis on *in vivo* methods for testing and prediction of oral dosage form performance. A wide range of factors and methods are considered in addition, such as imaging methods, perfusion models, models for predicting segmental/regional absorption, *in vitro in vivo* correlations as well as models to investigate the effects

Abbreviations: ABC, ATP-Binding Cassette; A_n, absorption number; API, active pharmaceutical ingredient; AUC, area under the curve; BCRP, breast cancer resistant protein; BCS, biopharmaceutic classification system; BDDCS, biopharmaceutics drug disposition drug classification system; BE, bioequivalence; CYP, cytochrome P450; D_0, dose number; D_n, dissolution number; DDI, drug–drug interactions; DSC, differential scanning calorimetry; EMA, European Medicines Agency; f_{abs}, fraction of dose absorbed; FaSSIF, Fasted state simulated intestinal fluid; FDA, Food and Drug Administration (USA); GI, gastrointestinal; GIT, gastrointestinal tract; GITT, GI transit times; GST, glutathione-S-transferase; HPβCD, hydroxypropyl-β-cyclodextrin; HPMC, hydroxypropyl methylcellulose; IMMC, interdigestive migrating motor complex; IR, immediate release; IVIVC, *in vitro in vivo* correlation; IVIVR, *in vivo in vitro* relationship; LC–MSMS, Liquid chromatography–tandem mass spectrometry; MAD, maximum absorbable dose; mDDI, metabolic drug–drug interactions; MR, modified release; MRI, magnetic resonance imaging; MRP2, multidrug-resistance associated protein 2; NMP, N-methyl-2-pyrrolidon; OrBiTo, oral biopharmaceutic tools; PBPK, physiologically-based pharmacokinetic; PD, pharmacodynamics; P_{eff}, effective permeability; PEG, polyethylene glycol; P-gp, P-glycoprotein; PK, pharmacokinetics; QbD, quality by design; SITT, small intestinal transit time; SLC, solute carrier superfamily; SLS, sodium lauryl sulfate; SULT, sulfotransferase; tDDI, transporter drug–drug interactions; TIM, TNO intestinal model; TPGS, D-α-tocopheryl polyethyleneglycol 1000 succinate; UGT, uridine 5'-diphosphate glucuronosyl transferases; XRD, X-ray diffraction.

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of excipients and the role of food on drug absorption. One goal of the authors was to clearly identify the gaps in today's knowledge in order to stimulate further work on refining the existing *in vivo* models and demonstrate their usefulness in drug formulation and product performance testing.

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Trade names used and their chemical names

Acconon E® Polyoxypropylene 11 stearylether or Polyoxypropylene 15 stearylether
Capmul PG8® Propyleneglycol monocaprylate
Cremophor RH40® (Kolliphor RH40®) Macrogolglycerol hydroxystearate
Cremophor EL® (Kolliphor EL®) Polyoxy 35 hydrogenated castor oil
Eudragit E® Butylated methacrylate copolymer
Imwitor 742® Caprylic/capric glycerides
Labrasol® Caprylocaproyl macrogol-8 glycerides
Methocel® Methylcellulose and hydroxypropylcellulose polymers
Miglyol® Triglycerides, medium chain
Pluronic® (Poloxamer) Ethylene oxide, propylene oxide block copolymer
Softigen® Macrogol 6 caprylic/capric glycerides
Solutol® Macrogol 15 hydroxystearate
Tween 20® (Polysorbate 20) PEG(20)sorbitan monolaurate
Tween 40® (Polysorbate 40) PEG(20)sorbitan monopalmitate
Tween 80® (Polysorbate 80) PEG(20)sorbitan monooleate

1. Introduction

Optimized and robust *in vivo* performance of an oral drug product is of crucial importance for its successful clinical application. Yet the lengthy drug development process provides little opportunity for optimization of the therapeutic product in humans. The subject of this review is a part of the Oral Biopharmaceutical Tools (OrBiTo) project within the Innovative Medicines Initiative (IMI) framework, a pre-competitive collaboration between pharma industry, academia and specialist technology companies. It aims to enhance understanding of how orally-administered drugs are absorbed from the gastrointestinal tract and to apply this knowledge to develop new *in vitro* tests and *in silico* models that will better predict the performance of oral formulations in humans. This review does not deal with drug absorption from the oral cavity and the esophagus although they represent parts of the GI-tract. In this review, *in vivo* methods for drug absorption, their comparative physiologies, correlations with *in vitro* methods (IVIVC), and applications for formulation/API/excipient characterization including food effects are covered. Attention has been paid to also point out the gaps in todays knowledge.

Pharmaceutical product characteristics, e.g. particle size, shape and physical form of the active pharmaceutical ingredient (API) in its dosage form are influencing its performance in the fasted and fed as well as in health and diseased states. Thus, API availability and its pharmacokinetics (PK) and/or –dynamics (PD) are highly dependent on the APIs presentation to the body by the formulation and the interaction with the changing local physiological conditions in the GI-tract (GIT). Such local conditions may change as a result of co-medication. For example, the use of proton pump inhibitors increases the gastric pH which may affect dissolution of acids and bases. Additional factors such as solubility, absorption, metabolism and disposition characteristics of the API come into play and may determine the variability of the PK and PD responses.

Biopharmaceutical factors related to the *in vivo* performance of the dosage form in the respective preclinical model such as pig, dog, rat, mouse and also in humans, i.e. GI-motility, GI-transit, mechanical stress, effects of food, enzymatic or pH-related degradation of API but also excipients, API release profile and API absorption in various GI segments, and the direct influence of some excipients on drug metabolism and transport are still insufficiently understood. Most of these factors have little or no impact for formulations of biopharmaceutic classification system (BCS) class I drugs, but are of greater influence for APIs from BCS classes II to IV, since their *in vivo* performance relies to a greater extent on the characteristics of their formulation. Therefore, it is obvious that for targeted, designed pharmaceutical products, the dosage form type, its composition in terms of identity and quantity of pharmaceutical excipients, the manufacturing process and the quality process parameters need to be defined and optimized. Quality by design (QbD) approaches require the definition of a design space for the raw material characteristics, the manufacturing process and its process conditions to assure predefined bioperformance of the manufactured product based on an appropriate design space.

Current biopharmaceutical tools in the pharmaceutical development rely on *in vitro* dosage form characterization, whole animal studies in mice, rats, dogs, pigs and/or monkeys and studies in healthy human subjects and sometimes in patients. While studies in human subjects can provide highly relevant information, they face various complexities including their justification by an Ethics Committee with additional limits in terms of throughput and cost. Therefore, *in vivo* studies in animals are sometimes preferred and human studies frequently are done to confirm and translate predictions to the human situation created from the other methods. Often animal studies yield species differences and pose the question of which animal species is most representative for humans. Underlying reasons for these species-specific findings include differences in anatomy and physiology of the GI tract as described in detail in the following sections. Not only might the species be less representative, but also the study setup like dosage regimen, amount water/meal, chewing, etc. does frequently not reflect the intended daily practical use of the dosage form in humans. Nevertheless, pharmaceutical development relies on these studies, since animals represent intact organisms necessary to simulate the complex interplay of drug dissolution, permeation and metabolism and they are used in legally mandatory toxicology studies and drug disease models such as achlorhydria. Furthermore it should be pointed out that the potential knowledge acquired from animal studies should always be measured towards the intrinsic value of the animal. Ethical issues in terms of animal sacrifice, discomfort and pain must always be considered. These aspects were acknowledged by Russel and Burch in their principle of 3Rs, Replacement, Reduction and Refinement (Russell and Burch, 1959). The essence of the principle to, replace the use of animals with alternative

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non-animal assays whenever possible, reduce the number of animals by optimized study designs and to refine the methodologies to minimize stress and pain is now internationally endorsed by the scientific community. Strong efforts in many disciplines have been made according to the 3Rs principle in the last decades and are for pharmaceutical investigations covered in the EU directive 2010/63/EU on the protection of animals used for scientific purposes.

Likewise *in vitro* methods have been used to predict effects in animals and in humans and, in order to demonstrate their predictability, *in vitro in vivo* correlations (IVIVC) have been recommended and are included in EMA and FDA guidelines. Thus, when robust and accurate predictability can be demonstrated by an *in vitro* method in conjunction with a validated IVIVC, a bioequivalence (BE) study can be waived on the basis of a dissolution test. But IVIVCs are frequently limited, to this point, to certain modified release (MR) dosage forms and often fail due to unknown reasons, which may either be attributed to non-physiologic (poorly biorelevant) *in vitro* test conditions such as volume and composition and static environment in the compendial dissolution tests that also do not take permeability and/or metabolism into consideration or to insufficient systems characterization, in terms of other presystemic factors that influence absorption and systemic availability of the API *in vivo*. The simulation programs applied today are fairly simplistic and may poorly reflect various physiological aspects important for GI drug absorption and often are not sufficient accurate (Poulin et al., 2011).

It is the concept of the 'integrated picture' reflected by the key biopharmaceutical parameters related to the API, its immediate release (IR) or MR dosage form and the human and animal systems characteristics that the authors strive to present in this review. This will assist in the definition of the current status and identify the gaps that are the focus of research performed in the OrBiTo project.

2. Human GI characterization

The basic design of the gut is a long muscular tube with specialized areas for digestion and storage, supplied by arteries and drained by veins and a lymphatic trunk, all supported in a sheath of connective tissue below the thorax, termed the mesentery. Functionally, the gut is divided into a preparative and primary storage region (mouth and stomach), a secretory and absorptive region (the midgut), a water reclamation system (ascending colon) and finally a waste-product storage system (the descending and sigmoid colon) as is illustrated in Fig. 1. Based on the luminal environment and the nature of the tissue change along the GIT, only the small intestine is structured to allow for maximal absorption. Important factors for GI drug absorption include the free luminal concentration of API, the effective area, interaction with luminal particles and transit time. A summary of key anatomical and physiological parameters of the human GIT is given in Table 1.

2.1. Stomach physiology

Stomach physiology and its impact on drug dissolution have recently been reviewed (Koziolek et al., 2013a,b). The stomach is divided into three functional parts. The fundus region acts together with the middle part of the stomach (corpus) as a storage compartment. In the distal part (antrum), food particles are milled, sieved and finally emptied through the pylorus. The size of the stomach depends largely on the filling status. Under fasting conditions, the stomach is mostly empty containing only approx. 10–50 mL of gastric juice as well as some gas. After meal intake, stomach filling volume may increase to 1 L or more, depending on the ingested

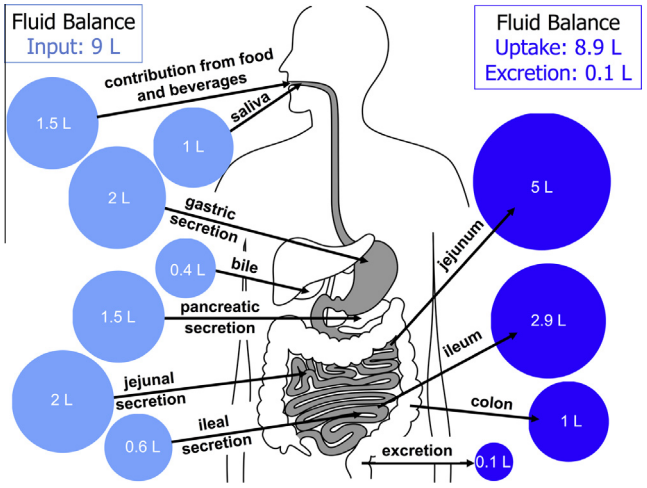

Fig. 1. Fluid balance in the human gastrointestinal tract. Adapted from P.E. Paulev, G. Zubieta-Calleja. Textbook in Medical Physiology and Pathophysiology: Essentials and Clinical Problems, second ed. Copenhagen, 2004 (ISBN 87-984078-0-5).

volume and individual physiology (Chial et al., 2002; Geliebter, 1988; Geliebter and Hashim, 2001).

The actual volume of the gastric content is the sum of meal volume, fasting gastric volume, cumulative saliva and gastric secretion minus gastric emptying (Goetze et al., 2009; Kwiatek et al., 2009). The total gastric volume has been shown to be larger than the consumed meal volume up to 3 h after intake of a light meal (Burton et al., 2005). During digestion, gastric juice is produced with a total daily secretion volume in the range of 2–3 L. In the fasted state, an unstimulated secretion rate of about 1 mL/min occurs which increases after meal intake to rates of 10 mL/min up to 50 mL/min (Versantvoort et al.). Another source of gastric filling volume is saliva with an also stimulation dependent flow rate of up to 10 mL/min and a total daily secretion volume of about 1–1.6 L per day (Engelen et al., 2003; Gaviao et al., 2004; Pedersen et al., 2002). Due to moderate peristaltic mixing, gastric contents are not homogenously distributed. Typically, a lipid layer is located on top of the gastric fluid due to the lower density of fat compared to that of water. However, subject posture and ingestion order influence the location of the lipid layer (Chang et al., 1968; Kunz et al., 2005). Solid high density particles accumulate in the more distal parts of the stomach due to their higher gravity, where they may be ground by a moderate antral milling activity. Gastric emptying occurs as a decanting process of the watery phase with small suspended particles and emulsion droplets (Keinke et al., 1984).

The flow properties of gastric contents range from Newtonian flow for pure water towards non-Newtonian, pseudoplastic flow behavior showing shear thinning, i.e. lower viscosity with shear forces acting on the contents in the presence of solid particles (Dikeman et al., 2006; Marciani et al., 2000; Mudie et al., 2010; Takahashi and Sakata, 2002, 2004). Estimated values for the viscosity of the gastric contents are in the range of 10–2000 mPa s (Abrahamsson et al., 2005).

Stomach motility is characterized by two different gastric motor patterns that originate from pacesetter cells located at the greater curvature of the corpus. In the fasted state, the interdigestive migrating motor complex (IMMC) occurs that enables the emptying of non-digestible objects from the gastric lumen during phases of high intensity with maximum pressures in the pyloric region of up to 300 mbar (Cassilly et al., 2008; Khosla and Davis, 1990). One IMMC front moves from the proximal stomach to the ileum every 1–2 h (Sarna, 1985; Vantrappen et al., 1977). The IMMC is interrupted by meal ingestion as the digestive motor

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Table 1
Comparison of the gastrointestinal tract of humans and animals with respect to anatomy and physiological parameters of relevance for drug absorption studies.

Parameter		Human	Canine	Pig Landrace (LR) Minipig (MP)	Rat	Mouse
pH fasted	Stomach	1–3.5 (Dressman et al., 1990; Hila et al., 2006; Simonian et al., 2005)	1.5–6.8 (values from various studies) (Arndt et al., 2013)	1.2–4.0 (Hossain et al., 1990) LR 0.3–1.7 (Oberle and Das, 1994) MP	4–5 (glandular region); 7 (anterior region) (Davies and Morris, 1993; Kararli, 1995)	4.0 (McConnell et al., 2008a)
	SI	6.0–7.0 (Duodenum) 6.0–7.7 (Jejunum) 6.5–8.0 (Ileum) (Brener et al., 1983; Ferraris et al., 1983; Lennernäs, 2007c) (46–48)	6.1–7.6 (de Zwart, 1999; Kalantzi et al., 2006; Sutton, 2004)	7–8 (Oberle and Das, 1994) MP	4.5–7.5 (Davis and Wilding, 2001; Lennernäs and Regårdh, 1993)	5.0 (McConnell et al., 2008a)
	Li	5.5–6.5 (Cecum) 5.5–7.5 (Colon asc.) 7.0–8.0 (Colon desc)	~6.5–unspecified dosing conditions (de Zwart, 1999)	n.a.	n.a.	4.7 (McConnell et al., 2008a)
pH fed	Stomach	3.0–6.0 (Kararli, 1995; Simonian et al., 2005)	Up to neutral – depends heavily on the pH of the ingested food, due to minimal gastric acid output	4.4 (Merchant et al., 2011) LR 3.6 (Oberle and Das, 1994) MP	3.8–5.0 (Davies and Morris, 1993)	3.0 (McConnell et al., 2008a)
	SI	5.0–5.5 (Duodenum) 5.0–6.5 (Jejunum) Similar to fasted (Ileum) (Brener et al., 1983; Davies and Morris, 1993; Persson et al., 2005)>4 h after meal: fasted pH	6.1 (Kalantzi et al., 2006) 5.5–7.2 (Duodenum/Ileum) (Sutton, 2004)	Duo: 4.7–6.1 Jej: 6.0–6.5 Ile:6.3–7.2 (Braude et al., 1976; Merchant et al., 2011) LR	6.5–7.1 (Davies and Morris, 1993)	4.8 (McConnell et al., 2008a)
	LI	n.a.	~6.5–unspecified dosing conditions) (de Zwart, 1999)	6.1–6.6 (Merchant et al., 2011) LR	6.6–6.9 (Davies and Morris, 1993)	4.5 (McConnell et al., 2008a)
Transit time fasted	Stomach	10–15 min ($t_{1/2}$) for liquids 0–2 h for indigestible solids (Brener et al., 1983; Davis et al., 1986)	Solution: 2–76 min Solid 7 × 20 mm: 1.2 h ($t_{1/2}$) (Reppas et al., 1991; Sutton, 2004)	1–28 days* (Hossain et al., 1990) LR	15–30 min ($t_{1/2}$) (Langguth et al., 1994) 5–65 min ($t_{1/2}$) (Maerz et al., 1994)	n.a.
	SI	3–4 (Davis et al., 1986)	60–111 min (Sutton, 2004)	<1–3 days* (Hossain et al., 1990) LR	3–4 h (Davis and Wilding, 2001; Lennernäs and Regårdh, 1993)	n.a.
	Li	8.0–18.0 (Davis et al., 1986)	Shorter than humans based on absorption data (Sutton et al., 2006) unspecified dosing conditions) and length (Kararli, 1995)	<1–3 days* (Hossain et al., 1990) LR	10–11 h based on a total GI transit time of 15 h (DeSesso and Jacobson, 2001)	n.a.
Transit time fed	Stomach	Liquid: Rapid but slower than the same liquid in fasted state (Brener et al., 1983) Digestible solids: Very rapidly for particles <2 mm (99% emptying in 0.5–3 h) (Davis et al., 1986) Rapidly for size <7–10 mm; larger particles held for many h (DeSesso and Jacobson, 2001)	Time to phase III activity: 5.4–13.3 h (Sutton, 2004)	Solution/pellets 1.4–2.2 tablet 1.5–6.0 (Davis et al., 2001; Wilfart et al., 2007) LR	n.a.	1 h (Padmanabhan et al., 2013)
	SI	3–4 (Davis et al., 1986)	Jejunal: 150–180 min (Sutton, 2004)	3–4 h (Davis et al., 2001; Wilfart et al., 2007) LR	n.a.	1–2 h (Hamada et al., 1999; Padmanabhan et al., 2013)
	Li	n.a.	Shorter than humans based on absorption data (Sutton et al., 2006) unspecified dosing conditions) and length (Kararli, 1995)	24–48 h (Davis et al., 2001; Wilfart et al., 2007) LR	n.a.	3 h (Padmanabhan et al., 2013)
Length SI		7 m (post mortem) (Dressman, 1986; Ferraris et al., 1983) 3.0–5.0 (*in vivo*) (Hofmann et al., 1983)	2.5–4.1 m (de Zwart, 1999)	840–900 cm (34–63 cm/kg) (Kurihara-Bergstrom et al., 1986; Suenderhauf and Parrott, 2013) MP 470–2000 cm (17–19 cm/kg) (Bergman et al., 2009; Merchant et al., 2011) LR	102–148 cm (Kararli, 1995)	40.2 cm (Ogiolda et al., 1998)
Length Li		1.5 (DeSesso and Jacobson, 2001)	Cecum: 8 cm Colon: 34–60 cm (de Zwart, 1999)	323 cm (11 cm/kg) (Glodek and Oldigs, 1981; McRorie et al., 1998) MP 436 cm (4.3 cm/kg) (Merchant et al., 2011) LR	26–26 cm (Kararli, 1995)	8.3 cm (Ogiolda et al., 1998)

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Table 1 (*continued*)

Parameter		Human	Canine	Pig Landrace (LR) Minipig (MP)	Rat	Mouse
Bile concentration	SI	2.0–10 mM (fasted) (Hofmann et al., 1983; Persson et al., 2005) 8.0 (fed) (Persson et al., 2005) 10–20 mM (after meal) (Hofmann et al., 1983)	Fasted state: Approx. 10 mM (values from various studies, (Arndt et al., 2013) 2.4–9.4 mM (Kalantzi et al., 2006) Fed state: 12.8–18.0 mM (Kalantzi et al., 2006) Most abundant is taurocholic acid (Holm et al., 2013b)	42–55 mM (Juste et al., 1983) LR	33.5–61.3 mM (fasted) (Staggers et al., 1982) 17–18 mM (fasted) (Kararli, 1995) Compared to man higher BS/PL ratio but PL concentration similar to man	n.a.
Metabolic activities	Phase I	CYP3A4, 2C9, 2C19, 2D6, 2J2 (see also Table 2)	Different than in humans (Haller et al., 2012)	See Table 3	CYP related activities (Takemoto et al., 2003) In general not correlated to humans	CYP1a1, 1b1, 2b10, 2b19, 2b20, 2c29, 2c38, 2c40, 2e1, 3a11, 3a13, 3a16, 3a25, 3a44 (Komura and Iwaki, 2008; Zhang et al., 2003)
	Phase II	UGT, SULT, GST	Different than in humans (Haller et al., 2012)	UGT, SULT, GST	β-Glucuronidase, sulfate conjugation, glucuronidation, N-acetylation In general not correlated to humans	UGT (Komura and Iwaki, 2011)
Major drug transporters		P-gp, MRP2, BCRP, PepT1, OATP	Peptide transporter-1 (PEPT1, SLC15A1, organic cation transporter-1 (OCT1, SLC22A1), BCRP), and multidrug resistance-associated protein 1 (MRP1, ABCC1) resemble the human tissue distribution (Haller et al., 2012)	P-gp, BCRP, MRP2, OATP	Similar transporter expression patterns as in humans (Cao et al., 2006)	P-gp (Holmstock et al., 2013)
Permeabilities		Reference	Higher than human for low permeability drugs (e.g. (Fotaki et al., 2005))	Less than in humans	Less than in humans, good correlation	Similar to human (Escribano et al., 2012)
Water volumes	Stomach	<50 mL (fasted) Up to 1 L (fed) (Chial et al., 2002; Geliebter, 1988; Geliebter and Hashim, 2001)	Similar to humans especially for dogs >20 kg (Martinez et al., 2002)	Wetmass: ~250 g (Merchant et al., 2011) LR	2.4 mL (Takashima et al., 2013)	0.37–0.71 mL (McConnell et al., 2008a)
	SI	Water pockets (Schiller et al., 2005)	No specific data–water flux in fasted upper GI is similar with humans (Reppas et al., 1991)	Wetmass: ~500 g (Merchant et al., 2011) LR	3.0–4.6 mL (Takashima et al., 2013)	0.81 mL (McConnell et al., 2008a)
	Li	Negligible (Schiller et al., 2005)	n.a.	wetmass: ~750 g (Merchant et al., 2011) LR	n.a.	0.6 mL (McConnell et al., 2008a)

n.a.: not available.
* Measured using nondisintegrating formulations.

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activity is initiated. The intensity of the gastric pressure waves is typically lower in the fed state than during phases of high intensity whilst fasting (Ouyang et al., 1989).

Little *in vivo* data on gastric flow is available due to the experimental difficulties for these measurements. However, Boulby et al. observed peak velocities of 2–8 cm/s (Boulby et al., 1999). Computer simulations based on computational fluid dynamics have also been performed but reported a rather broad range of estimated values (Ferrua et al., 2011; Pal et al., 2004). Under postprandial conditions liquids may probably also be cleared within a few min from the stomach due to a mechanism called "Magenstrasse" (Pal et al., 2007).

As for hydrodynamics, data on intragastric mechanical conditions are highly variable. It seems, however, that the antral grinding forces represent the highest shear forces acting on solids in the fed stomach with grinding force values in the range of 0.2–1.89 N (Kamba et al., 2000; Marciani et al., 2001a).

During digestion only liquids and small suspended particles are delivered to the small intestine whilst larger particles are retained by gastric sieving (Meyer et al., 1981). Due to the diversity of the relevant food parameters it is not possible to define a clear cut-off size (Khosla and Davis, 1990; Newton, 2010; Siegel et al., 1988).

Liquids are emptied according to first-order kinetics with emptying rates that are influenced by both caloric content and meal composition. Ranges are reported from 2 to 4 mL/min, however, initial emptying rates may reach values of up to 10 to 40 mL/min (Faas et al., 2001; Indireshkumar et al., 2000; Kong and Singh, 2008; Kwiatek et al., 2009). Comparable high emptying rates are also observed after ingestion of water (non-caloric liquids) under fasting conditions (Oberle et al., 1990). Solid particles are emptied according to a biphasic pattern.

2.2. Intestinal surface area

The surface area of the gut is commonly regarded as a long muscular tube, which is increased by foldings, and by small intestinal villi and microvilli. Based on static morphology, several workers have calculated the apparent mucosal surface area of the small intestine after removal, fixation and staining to be approximately 2.2 m^2 (Wilson, 1967). These histological measurements could not take account of the microvilli and their presence complicates the estimation of surface area since on scanning electron microscopy they are seen to be a tightly packed array. It appears that for nutrition, there is an excess capability and only the top of the villus may be utilized. In addition, the simple static concept is misleading. The foldings change dynamically with transit of food and the microvilli break off to form mixed micellar phases near the apical boundary. The effective surface area is highly dynamic and is affected by nutritional status, exposure to noxious agents and by the luminal viscosity.

2.3. The pH of the GIT

In the fasted state the gastric pH value of healthy adults is reported to be within pH 1 to pH 3 (Dressman et al., 1990; Hila et al., 2006; Simonian et al., 2005). In elderly patients and also as a function of ethnic difference, various degrees of achlorhydria have been reported (Charman et al., 1997). After meal intake, the pH of the gastric content is increased to various degrees. For instance, after intake of the FDA standard breakfast, the maximum stomach pH was reached within the first 5 min and pH decreased to values below pH 3 not before 56 ± 42 min. Due to regional differences in the presence of acid secreting glands, pH gradients in the stomach contents have been observed (Simonian et al., 2005).

Although compendial estimates of the pH of the stomach simulated by simple media have varied over the years from pH 1 to pH 1.2, it is generally accepted that bicarbonate secretion is important, raising the pH in the sub-mucin layer. The contribution of occasional duodenal-gastric reflux must also not be neglected as it produces transient neutralisation as illustrated in Fig. 2. Even within a subject, it can also be appreciated that a wide range of gastric pH with time occurs. The data also reflect the observation that in the stomach, the pH in the fundus will typically be one pH unit higher than in the pyloric antrum. In the fed stomach, the sampling device can find itself in pockets of acid or in the food mass (Vo et al., 2005). Twenty-four h radio-telemetry data show the daily excursions in pH, with very short periods at pH 1, transient rises to up to pH 5 and slow recovery to baseline as illustrated in Fig. 3. The daily intake of food causes rises in pH, with fatty meals causing a sustained rise in proximal gut pH, which may be important if a heavy meal is taken at night. This occurs rarely in clinical trials but may be fairly common in the western world in the general population. Treatment with proton pump inhibitors reduces the number of pockets of acid (detected by pull through) and increases their pH (Vo et al., 2005). Increased gastric pH due to therapy with proton pump inhibitors or H_2 receptor blockers may be of concern for enteric coated dosage forms and is also discussed as a possibly quite often overseen source for a reduced bioavailability of drugs with a strongly pH dependent solubility profile. For example, this has been recently reviewed for a number of oral anticancer drugs (Budha et al., 2012). Besides the changes in gastric pH, proton pump inhibitors also reduce stomach secretions (Babaei et al., 2009; Nishina et al., 1996). This anti-secretory effect may also contribute to a reduced bioavailability of poorly soluble compounds.

2.4. GIT transit and motility

In normal physiology, a balance exists between propulsive, peristaltic movements and mixing contractions. This is controlled by signalling between external nerves, especially the vagus, by intestinal short-range pathways and through the plexii. Local responses also occur and may cause spasm.

The fasting and fed patterns of GI motility are distinct and have been examined extensively *in vivo* (Szurszewski, 1969). In the fed mode, contractions travel down the wall of the stomach, originating below the fundus and forming an annular ring, the pyloric cylinder. Towards the pylorus, the walls collapse, squeezing the contents through a partially closed sphincter and causing retropulsion of larger debris back into the body of the stomach. The mechanism sieves the contents, retaining larger objects for further grinding and is a major determinant of the gastric emptying and onset of drug absorption for any pharmaceutical solid dosage form.

Gastric sieving is also the cause for the retention of large non-disintegrating dosage forms in the stomach which lasts until complete emptying of caloric contents and the appearance of the IMMC. A clear cut-off dimension for the particle size which may allow emptying from the stomach during digestion cannot be given (Newton, 2010). A particle size below 2 mm is usually considered as small enough for emptying during fed state motility. Tablets with a size of 3 mm have clearly been demonstrated to be retained with food (Podczeck et al., 2007). Disintegrating objects, near the sphincter are emptied as a series of pulses. This wave of contraction travels from the stomach to the terminal ileum and then vanishes.

In the fasted state IMMC consists of four phases that are characterized by distinct intensity and duration. The IMMC front moves from the proximal stomach to the ileum and restarts afterward. These cycles repeat until food is ingested and a complete cycle lasts for approximately 1–2 h. Most of all the powerful contractions that are characteristic for phase III activity (the so-called housekeeping waves) serve as a general cleansing allowing the emptying of large

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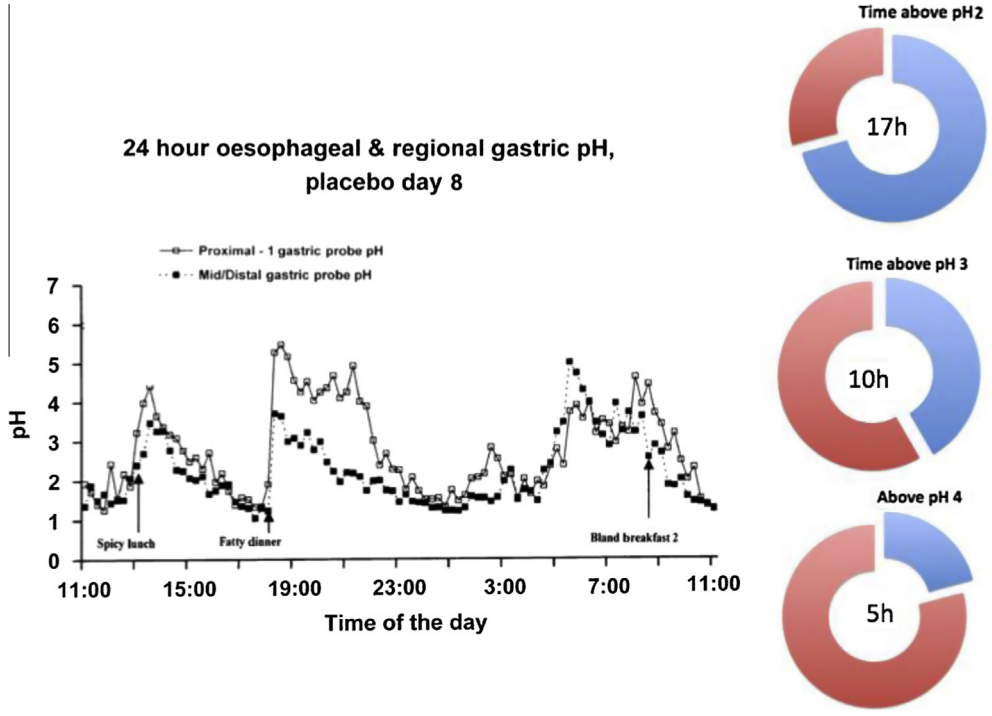

Fig. 2. Distal and proximal 24 h pH-monitoring in human.

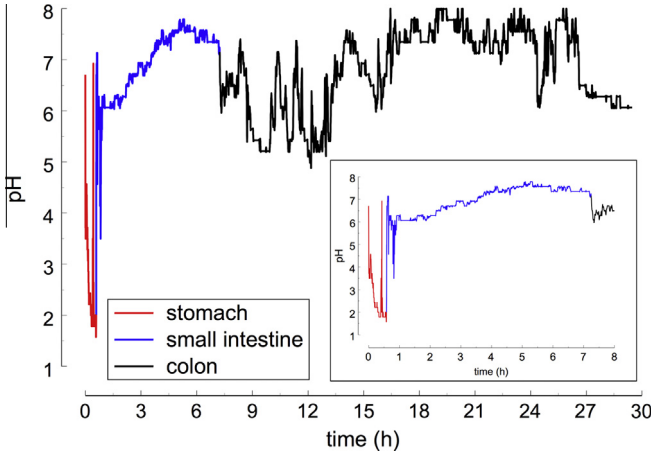

Fig. 3. pH-profile recorded by a telemetric IntelliSite capsule in a healthy volunteer during GI transit. Capsule ingestion occurred under fasting conditions. (Data kindly provided by Medimetrics.)

non-digestible solids like enteric coated tablets or monolithic extended release tablets from the stomach (Cassilly et al., 2008; Khosla et al., 1989). The stomach is emptied for small particles under fasted state with emptying times from approx. 15 min to more than 3 h and can be grouped into immediate and rapid, delayed but rapid, delayed and slow, and interruptive emptying (Locatelli et al., 2009).

As discussed in several reviews, the GI luminal conditions change as a function of the specific GI site (Varum et al., 2010; Weitschies et al., 2010; Wilson, 2010). The intraluminal pressure following the passage through the pyloric sphincter and ileocaecal valve may reach values of up to 300 mbar (Cassilly et al., 2008; Dinning et al., 1999). Furthermore, similar high pressure amplitudes are also recorded in the colon (Rogers et al., 1989). Such high pressure events can result in an altered *in vivo* drug release profile or even possibly affect release mechanisms from MR dosage forms. The small intestinal transit time (SITT) is in the range of 3–4 h and appears not to be dependent upon the dosage form (Davis et al., 1986). However, this value is probably a reflection of the typical feeding regimen used in clinical trials as small intestinal transit is triggered by food intake via a mechanism known as gastro-ileocaecal reflex (Fadda et al., 2009; Schiller et al., 2005). Movement of dosage forms through the small intestine is characterized by typically short episodes of transport where peak velocities of up to 50 cm/s may be reached (jet propulsion) and phases of rest (Weitschies et al., 2005). Typically, dosage forms spend most of the total transit time at rest in the small intestine, typically in the terminal ileum (McConnell et al., 2008b). Under fasting conditions, dosage forms are not necessarily in permanent contact with intestinal water (Schiller et al., 2005).

Interestingly, small particles move faster than large particles in colon and due to the extremely variable composition and viscosity of colonic contents, hydrodynamic properties are extremely difficult to predict (Abrahamsson et al., 1996; Arkwright et al., 2013; Follonier and Doelker, 1992; Wilson, 2010).

2.5. Presence of GI fluids

Body fluid balance is primarily regulated by renal mechanisms, by which the body conserves electrolytes and achieves acid–base balance. GI fluids are produced by saliva, gastric and intestinal secretions, pancreatic secretions and ingested fluids. The small intestine is a very efficient absorber of water, which has a high effective permeability (P_{eff}) in the jejunum *in vivo*, approximately 2×10^{-4} cm (Fagerholm et al., 1995). The half-time of gastric emptying of water is approximately 10–15 min or less. After emptying into the small intestine, water is quickly taken up into the systemic circulation. In magnetic resonance imaging (MRI) images, residual water can only be seen in a few pockets along the small intestines (Schiller et al., 2005). Free water is rarely seen in the colon (Schiller

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et al., 2005). Also in relation to postprandial response, bile, bicarbonate, electrolyte composition, etc. play an important role in dosage form disintegration (coating), dissolution, solubilization and absorption of the API.

2.6. Abundance of drug metabolizing enzymes

It has been proposed that the intestinal mucosa is the most significant extra-hepatic site for drug metabolism (Lin and Lu, 2001). The intestine possesses the potential to considerably impact drug bioavailability (Yang et al., 2007). In order to predict first-pass effects in the intestinal wall, it is necessary to characterize the region-specific expression of enzymes along the GIT. This is particularly relevant to the development of mechanistic physiological-based intestinal models that are capable of assessing the contribution of these enzymes to intestinal metabolism, portal vein availability and intestinal-based DDI (Jamei et al., 2009; Pang and Chow, 2012). Given that the variability in metabolism can be considerable between individuals (Lampen et al., 1996, 1995), identifying the basis of these differences, i.e., the variability in abundance and or activity of the enzymes responsible for the metabolism of a drug may be crucial (Gertz et al., 2011; Proctor et al., 2004; von Richter et al., 2004).

The enzymes most extensively expressed in the small intestine are the cytochrome P450's (CYPs) (Paine et al., 2006; Thelen and Dressman, 2009). Studies of the human intestine have shown that the CYP isoform with the highest specific content was CYP3A4. It has been reported that average CYP3A4 abundances (65–70 nmol/total gut) incorporated into minimal intestinal models, such as the Q_{gut} model, were appropriate (Yang et al., 2007). However, the differential expression of CYP3A along the length of the small intestine (Table 2) in a fully mechanistic compartmental model is crucial for a development of any MR dosage form (Darwich et al., 2010; Jamei et al., 2009).

There are conflicting views of the expression and functional relevance of the metabolic contribution of CYPs in the colon (Canaparo et al., 2007; Lennernäs, 2007b). Canaparo et al. suggested that CYP3A isoforms are expressed in the colon. But an absence of CYP3A4 expression in certain samples has been demonstrated within this study, highlighting the potential variability for CYP3A4 expression in the colon between individuals.

In addition to the CYP3A family, the CYP2C, 2D and 2J family of enzymes are also expressed in the gut mucosa (Paine et al., 2006; Zhang et al., 1999). The relative contributions to the overall intestinal CYP content of (CYP3A4, 2C9, 2C19, 2D6, 2J2) were measured in 31 small intestinal donors (Paine et al., 2006), with details of region-specific expression provided in (Table 2). Reports utilizing liquid chromatography–tandem mass spectrometry (LC–MSMS) proteomic techniques to determine CYP abundance, are at present confined to measurements undertaken in Beagle dog intestinal regions (Heikkinen et al., 2012). Therefore, there are opportunities to expand on this technique to enhance our understanding of absolute protein abundance of numerous CYP isoforms in man.

The intestinal expression of intestinal phase II enzymes; uridine 5′-diphosphate glucuronosyl transferases (UGT), sulfotransferase (SULT) and glutathione-S-transferase (GST) also deserve consideration (Coles et al., 2002; Ritter, 2007; Trdan Lusin et al., 2011). Notably, there are fewer studies characterizing the expression and activity of phase II enzymes in the intestine as compared to CYPs. The review by Ritter (2007) highlights the interest towards studies utilizing mRNA-gene expression of UGT's in numerous intestinal segments (Ritter, 2007). Ritter asserts that the lack of specific antibodies, or specific probe substrates for individual UGT isoforms, in addition to the difficulty in purifying/synthesizing these full length recombinant membrane anchored proteins (Milne et al., 2011), hampers the characterization of quantitative protein activity relationships in tissues. Absolute protein abundance quantitation of intestinal UGT's by LC–MSMS proteomic techniques have been used to quantify numerous UGT isoforms in intestinal microsome samples (Harbourt et al., 2012; Smith et al., 2011). However, the levels of these enzymes along the length of the intestine are yet to be determined.

The quantification of absolute levels of SULT enzymes in the intestine is limited to a single documented study (Riches et al., 2009). Determination of SULT expression in cytosolic fractions from duodenal samples ($n = 6$) was undertaken by quantitative immunoblotting and identified 3 out of 5 SULT isoforms under study (SULT1A3, 1B1 and 1E1) and found that their abundance in the duodenum was greater than that for liver, kidney and lung cytosolic fractions. Further data for the expression of SULT's in other intestinal regions is yet to be established.

Additional enzymes such as epoxide hydrolase and aldehyde oxidases have been shown to be expressed in the small intestine (de Waziers et al., 1990; Moriwaki et al., 2001). Characterization of their abundance and activity in human intestinal cytosolic or S9 fractions will be required to facilitate the development of strategies in order to predict the impact of the activity of these enzymes on bioavailability.

Determining the transient levels of an enzyme is critical for the accurate prediction of DDI's involving mechanism (time)-based inhibition and induction. Enzyme levels are governed by the balance of the processes of *de* novo protein synthesis and degradation and thus, when these processes are in equilibrium, enzyme levels are at steady-state (Yang et al., 2008). This balance may be disturbed, for example, when the rate of enzyme synthesis with a concomitant absence of change or a decrease in degradation rate, leads to a rise in enzyme levels ('induction effect'). There are a host of *in vitro* and *in vivo* methods to determine enzyme turnover in

Table 2
Segmental distribution of intestinal CYP enzymes in human.

Enzyme	Duodenum	Jejunum I	Jejunum II	Ileum I	Ileum II	Ileum III	Ileum IV	Total intestinal abundance (nmol)
Segmental abundance (nmol)								
CYP2C9	1.78	3.51	3.51	1.02	1.02	1.02	1.02	12.9
CYP2C19	0.21	0.41	0.41	0.12	0.12	0.12	0.12	1.5
CYP2D6	0.11	0.22	0.22	0.06	0.06	0.06	0.06	0.8
CYP2J2	0.19	0.38	0.38	0.11	0.11	0.11	0.11	1.4
CYP3A4	9.11	18.03	18.03	5.26	5.26	5.26	5.26	66.2
CYP3A5	3.38	6.7	6.7	1.95	1.95	1.95	1.95	24.6

Intestinal CYP abundances are from Paine et al. (2006) and Paine et al. (1997) (Paine et al., 2006, 1997). In Paine et al. (2006), CYP abundances were relative expressions, therefore the abundances of CYP2C9, 2C19, 2D6, and 2J2 were calibrated against CYP3A total intestinal abundances.
The expression of the active CYP3A5 enzyme is phenotypic and dependent on an individual carrying at least one *CYP3A5∗1* allele. Within a Caucasian population, this occurs at a frequency of approximately 10–20% (Wrighton et al., 1989). There is evidence that there is also a correlation between the abundance of CYP3A4 and CYP3A5 in liver, however, a reciprocal relationship has yet to be verified in gut samples (Barter et al., 2010).

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hepatic systems (Yang et al., 2008). For the intestine, the situation is different because enterocyte stem cells arising from the crypt base migrate towards the villus tip, maturing and differentiating into fully functional enterocytes as they migrate. This migratory process can take between 1 and 10 days and is likely to be more rapid than enzyme turnover. Thus, turnover is likely to be governed by the enterocytes sloughing into the intestinal lumen rather than the intrinsic turnover of the enzyme. Enterocyte CYP turnover information can be estimated indirectly in studies administering grapefruit juice. Oral ingestion of grapefruit juice leads to a selective and irreversible inhibition of intestinal enzymes without affecting the hepatic enzymes (Schmiedlin-Ren et al., 1997; Won et al., 2012). Studies using this design with subsequent oral dosing of a probe substrate such as midazolam have been utilized to estimate a CYP3A enterocyte turnover half-life of 23 h (Greenblatt et al., 2003).

There is significant scope to expand our current knowledge of Phase I and II enzyme abundances in the gut. Further data should be obtained on the region-specific expression of Phase I and II enzymes, and the development of quantitative immunoblot and LC–MSMS proteomic techniques. This will enhance knowledge within this field, particularly where isoform specific antibodies or full length protein standards are not available. As more studies with increasing sample numbers become available analyses will focus on evaluating the inter-individual variability in abundance, together with defining abundance–activity relationships to provide more mechanistic approaches for *in vitro–in vivo* extrapolation (IVIVE).

2.7. Abundance of membrane drug transporter

Passive membrane diffusion processes, where molecules pass across membranes driven by concentration gradients play a significant role in the absorption of many drugs (Lennernäs, 2007b; Sugano et al., 2010). Yet, there is considerable evidence that many drugs can interact with transporter proteins expressed in enterocyte membranes along the intestine to either facilitate or reduce absorption rate (Varma et al., 2010). There is therefore a necessity to accurately gauge the intestinal region-specific expression of these transporters in order to predict the potential impact of these transporters on the GI drug absorption and dosage form development (Giacomini et al., 2010).

Epithelial cells including enterocytes are polarized and contain two functionally distinct membrane domains at either pole of the cell. The apical (luminal) membrane contains specific constituents that are structurally and functionally distinct to those at the basal (serosal-blood) side at the opposite pole of the cell. This includes the differential expression of transporter proteins in each membrane. The most thoroughly characterized transporter proteins are those that are members of the ATP-Binding Cassette (ABC) superfamily. Transporters such as P-glycoprotein (P-gp, MDR1, ABCB1), multidrug-resistance associated protein (MRP2, ABCC2) and breast cancer resistant protein (BCRP, ABCG2) are expressed on the apical membrane of the enterocyte. They transport drugs against the prevailing concentration gradient by binding them from the intracellular milieu or inner membrane leaflet and secreting (effluxing) these molecules into the intestinal lumen resulting in an absorption limitation (Aller et al., 2009). The impact of intestinal efflux transporters is expected to be at its greatest when the drug's passive diffusion across the apical membrane enterocyte is low (Darwich et al., 2010). In the clinical setting, the β_1-adrenoceptor antagonist talinolol demonstrated a dose-dependent increase in systemic exposure after oral administration in humans. *In vitro* mechanistic studies determined that the likely mechanism for this phenomenon was the interaction with P-gp (Wetterich et al., 1996). As the dose increases, the efflux function of P-gp reaches saturation, giving rise to the non-proportional increase in exposure by increasing drug transfer across the enterocyte apical membrane. In addition to ABC transporters, transporter proteins belonging to the solute carrier superfamily (SLC) operate to facilitate transport across the apical and basal enterocyte membranes by binding and co-transporting counter ions by symport or antiport mechanisms (Grandvuinet et al., 2012; Koepsell et al., 2007). The impact of SLC transporters on drug absorption is dependent on their membrane location and the direction into which they operate, i.e. where they transfer the substrate across the membrane in which they reside. For example, pro-drug strategies have utilized transporter function to enhance drug absorption by targeting the SLC intestinal uptake transporter (oligopeptide transporter PepT1). This was shown to augment drug absorption and increase bioavailability (Varma et al., 2010; Weller et al., 1993; Steffansen et al., 2005). Intestinal transporter–substrate interactions can be complex with flux mediated by transporters on both membrane poles bi-directionally as demonstrated for estrone-3-sulfate (Rolsted et al., 2011).

For many years there was a reliance on determining transporter protein expression by semi-quantitative immunoblotting or quantitative mRNA-gene expression approaches. These studies have employed blot densitometry techniques to evaluate relative expression differences between samples after calibration against a reference 'housekeeper' protein. Meta-analyses evaluating the regional heterogeneity of intestinal transporter expression from multiple literature sources, using immunoblot densitometry and mRNA-based expression, have been undertaken to incorporate expression-based transporter functionality into mechanistic intestinal models (Badhan et al., 2009; Bolger et al., 2009; Darwich et al., 2010; Harwood et al., 2013). It is common when using relative expression techniques to normalize the expression of transporters along the intestine to a single reference compartment, *i.e.* the proximal ileal or jejunal segments (Bolger et al., 2009; Darwich et al., 2010; Harwood et al., 2013). It appears there is no consensus as to the relationship of the regional-specific P-gp expression implemented across these models, which is likely to result from the data and statistical methodologies incorporated into the meta-analyses. In addition, the usage of mRNA-gene expression assumes a direct correlation to protein activity, which may not be the case for certain transporters (Berggren et al., 2007).

The reliance on relative expression techniques to the quantitation of transporter expression owes much to the challenges in purifying full length recombinant integral membrane proteins, and the lack of development of antibodies specific to transporter isoforms to act as standards for quantitative immunoblotting and enzyme linked immunosorbent (ELISA) assays (Ohtsuki et al., 2011). Over the last several years, development of targeted proteomic techniques has enabled the absolute quantification of integral membrane proteins such as transporters, using 'heavy' labeled isotopes as internal standards (Kamiie et al., 2008; Li et al., 2008). Two recently published studies have utilized these techniques to quantitate transporter abundances in 5 jejunal and 7 ileal mucosal samples (Groer et al., 2013; Oswald et al., 2013). The abundance of PepT1 was shown to be substantially higher than OATP2B1, P-gp, MRP2 and BCRP, however data from a greater number of samples is required to confirm regional-specific expression of these transporters. In addition, quantitative immunoblotting techniques have been employed to measure the absolute abundance of P-gp, MRP2 and BCRP in frozen duodenal tissues obtained from a human tissue bank (Tucker et al., 2012). In these assays, the mucosal surface was scraped and subsequently homogenized with an ensuing differential centrifugation procedure. P-gp, MRP2 and BCRP in total membrane fractions from 14 samples were measured by quantitative immunoblotting using s-tagging technology as an internal standard (Karpeisky et al., 1994). Within the duodenal membrane

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fractions, the transporter abundances in order from highest to lowest were; BCRP > P-gp > MRP2. This is in conflict with duodenal expression data from meta-analyses incorporating data using relative expression techniques that are normalized to the proximal jejunum (Harwood et al., 2013). These analyses show a reversal of expression in the order: MRP2 > P-gp > BCRP. The accurate implementation of expression data within intestinal models is critical to ensure the models proximity to the *in vivo* milieu to provide the basis for predicting the impact of transporters on ADMET.

There is a need to undertake further studies quantifying the region-specific abundances of a variety of ABC and SLC transporters that reside on the apical and basal enterocyte membranes and their variability between individuals in numerous populations. In addition, it would also be valuable to quantify the transporters OST-α and OST-β (OST-A & OAT-B) located on the basal enterocyte membrane (Ballatori et al., 2005; Grandvuinet and Steffansen, 2011) and cadherin-17/human peptide transporter 1 (CDH17, HPT1) which functions as a peptide transporter (Dantzig et al., 1994).

Many fundamental questions remain to be elucidated regarding accurately quantitating the levels of these proteins within tissues. Loss of proteins throughout practical workflow is inevitable. Strategies to counter these losses, or the use of recovery factors, to estimate the 'true' or 'intrinsic' tissue abundance levels could be utilized. For intestinal samples, a reasonably pure enterocyte yield is essential as contamination with connective tissue, i.e. lamina propria, and underlying submucosal tissue will dilute the sample. This is likely to lead to under-estimation in transporter abundance. Moreover, expression of transporter proteins in any intestinal layers or red blood cells other than the enterocytes will lead to contamination and may bias abundances. It has been speculated that different methods to obtain membrane fractions may be subject to their digestion and thus quantitation may lead to differences in abundance levels between studies (Prasad et al., 2013). Equally, the protocols employed to reduce, alkylate and digest proteins or the internal standard peptides used as surrogates for protein quantitation could influence the endpoint quantitation (Balogh et al., 2012). Therefore, it has been proposed that to elucidate whether there are any methodological biases leading to differences in endpoint abundances, matched protein samples should be processed across laboratories using their own in-house techniques where valid comparisons can take place (Rowland Yeo et al., 2013).

3. Canine GI characterization

3.1. Anatomical considerations

Detailed description of the anatomy of the canine GI tract can be found in various previously published reviews (de Zwart, 1999; Kararli, 1995) and a summary is provided in Table 1. The canine stomach is anatomically similar to that of humans, e.g., volume of 0.5–1 L (living beagle) (Martinez et al., 2002). Dogs possess a well developed small intestine, which is consistent with a diet that is low in fiber but high in fat and protein, and a relatively simple colon. Major differences, compared with humans, include the shorter small intestine, especially for small dogs, and the much shorter large intestine, about one-fourth of human colon (de Zwart, 1999; Kararli, 1995).

3.2. GI characteristics

Some GI characteristics are presented in comparison with data from other species in Table 3. An extended review on canine GI physiology is available (Smeets-Peeters et al., 1998).

Table 3
Presence of CYP isoenzymes in landrace and Göttingen minipig (modified from Helke and Swindle, 2013 (Helke and Swindle, 2013).

Human CYP	Landrace	Göttingen minipigs
1A1	+	
1A2	+	+
2A6	+	+
2B6	+	
2C9	+	–
2D6	+	–
2E1	+	+
3A4	+	+

+: indicated presence/activity of enzyme.
–: indicates no presence/activity of enzyme.

Canine gastric pH in the fasted state varies along the length of the stomach. Anterior gastric pH has a pH of 5.5 and drops to 3.4 in the posterior stomach (Smith, 1965). Perhaps it is important to mention that the most effective way to achieve a consistently low gastric pH in fasting dogs is to administer 0.1 mol/l HCl-KCL buffer 15 min before the dosage form. Similarly, to elevate the gastric pH reproducibly (in the fed state), omeprazole 1 mg/kg should be administered intravenously at least 90 min before oral administration of the dosage form (Polentarutti et al., 2010).

With regards to the fasted small intestine, all along the length of the small intestine the pH increases from pH 6.2 to about 7.5 (Kararli, 1995). This was confirmed by Kalantzi et al. (2006) who reported a fasted pH of 7.1. The buffer capacity has been measured 1.4–4.2 mM/pH and the osmolarity 62–207 mOsmol/kg, both lower than in humans (Kalantzi et al., 2006). The most abundant bile salt is taurocholic acid (Holm et al., 2013b). The phospholipid in the canine intestine contains 94.5% phosphatidylcholine and 5.5% phosphatidylethanolamine (Alvaro et al., 1986).

The type and pattern of contractions of the canine GIT in the fasted state are similar to those of humans. Phase III (housekeeping wave) of the IMMC lasts for about 20 min in both species and occurs every 100–110 min (Itoh and Takahashi, 1981; Yamada et al., 1995). In contrast, agitation intensity is higher in the GIT of dogs (Katori et al., 1995). In the fed state, the pattern of contractions in jejunum is similar although probably more intense (Schemann and Ehrlein, 1986).

Gastric emptying of liquids in the fasting state has been reported to be similar or faster compared with those in humans (Ehrlein and Prove, 1982; Gupta and Robinson, 1988; Hinder and Kelly, 1977; Reppas et al., 1991). In the fed state, the gastric emptying rate of liquids is slower compared to humans (Nishiyama et al., 1996). The gastric emptying of solids in the fasting state is size-dependent like in man and occurs at similar or faster rates (Aoyagi et al., 1992; Gruber et al., 1987). Solid meals are emptied at slower rates than in humans (Dressman, 1986; Meyer et al., 1981; Meyer et al., 1979). Transit times of the small and large intestine vary with the size of the dog. In beagles, they are about half of that of humans (Davies and Morris, 1993).

Information on intraluminal metabolic activity in the canine GIT has been very limited (de Zwart, 1999; Martinez et al., 2002). A recent study showed that the degradation of three ester prodrugs in jejunal fasted state contents collected from Labradors and healthy humans was similar (Borde et al., 2012). However, the enzymatic capacity of luminal contents in dogs was higher than in humans, which is in line with the higher protein levels measured in the canine luminal contents. Also, compared to the activity in luminal contents, the hydrolase activity in small intestine microsomes seemed to be lower in dogs but higher in humans (Borde et al., 2012).

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3.3. Intestinal permeability and metabolic activity

It has been estimated that the unstirred layer thickness for rapidly absorbed compounds is similar in dogs and humans (of the order of 40 μm) (Fagerholm and Lennernäs, 1995; Levitt et al., 1990). Differences in the available surface area and in the tightness of the junctions between epithelial cells have been considered to be important for low permeability compounds (Fotaki et al., 2005; He et al., 1998). There is some evidence for improved drug permeability through the canine small intestine as compared to human intestine which has been related to these factors (Sutton, 2004). However, good correlation of the relative bioavailabilities of 11 compounds administered to the dog and human colon have been observed (Sutton, 2004).

To date, the distribution of metabolic enzymes and membrane transporters in dogs has not been comprehensively investigated. Relevant studies have mainly focused on the expression and catalytic activity of metabolic enzymes in the liver (Kyokawa et al., 2001; Mills et al., 2010). Less is known about the intestinal expression distribution of CYPs, UGTs, membrane transporters as well as their substrate specificities and variability in expression compared with that in humans (Bock et al., 2002; Conrad et al., 2001; Fraser et al., 1997; Locuson et al., 2009; Mealey et al., 2008; Turpeinen et al., 2007).

According to a recent study (Haller et al., 2012), the gene expression pattern of five drug transporters in the liver and along the intestine of beagle dogs has a number of differences compared with human data. In particular, the tissue distribution of CYP isozymes and P-gp appears to be markedly different in dogs compared with humans, whereas UGT1A6, peptide transporter-1 (PEPT1, SLC15A1), organic cation transporter-1 (OCT1, SLC22A1, BCRP), and multidrug resistance-associated protein 1 (MRP1, ABCC1) more closely resemble the human tissue distribution (Haller et al., 2012).

3.4. Gall bladder emptying and lymphatic transport

In the fasted state, canine gallbladder shows brief alternating excursions of filling and emptying with the number of emptying events exceeding the filling events during phase II of IMMC (Abiru et al., 1994). In the fed state, the intensity of gallbladder contractions is dependent on the ingested calories and meal lipid content (Romanski and Slawuta, 2003). The *in vivo* lymph cannulated canine model has been described and used to study intestinal lymphatic targeting and transport. Khoo et al. (2001) have proposed a triple-cannulated conscious dog model, which allows sampling from the thoracic duct lymph as well as portal and systemic blood (Khoo et al., 2001). In contrast to other animal models, the dog model allows administration of dosage forms that are of a size relevant for human administration, and it facilitates to study the effects of fed versus fasted states on drug absorption. However, the higher concentration of bile acids in the GI chyme should be recognized (Persson et al., 2005).

3.5. Concluding remarks and gaps to be filled

Characteristics of the canine GIT, especially the luminal characteristics of large dogs (body weight of about 30 kg), show various similarities with man. The most important distinctions from the human GIT relate to the higher bile concentrations and higher solubility/dissolution of BCS Class II drugs, to the higher absorption of BCS class III drugs, and to differences in colonic characteristics (personal communication Abrahamsson; Persson et al., 2005). There are also indications that the intersubject variability in regard to GI characteristics is larger in dogs than in humans (de Zwart, 1999). A more thorough understanding of enzyme (both luminal and mucosal) and transporter activities in dogs, would improve the usefulness of this model in drug absorption and API/formulation studies.

4. Pig GI characterization

4.1. Introduction

Pigs are considered a translational model in biomedical research because of anatomical, physiological and biochemical similarities to humans (Puccinelli et al., 2011; Swindle, 2007; Swindle and Smith, 1998; Tissot et al., 1987). Pigs and in particular mini-pigs have therefore become increasingly popular as an alternative species in drug development (Bode et al., 2010; Ganderup et al., 2012; Helke and Swindle, 2013; Swindle et al., 2012). However, in the literature there is a disagreement on the potential use of pigs as an *in vivo* model for drug formulation research and development, which needs further exploration. In order to understand in which circumstances pigs should be considered, this section includes a description of pig GI physiology, metabolism and membrane transporters and the similarity/dissimilarity to humans. There are several breeds of minipigs, such as the Yucatan and Göttingen, where Göttingen minipig is the most frequently used pig strain in contemporary pharmaceutical literature (Simianer and Kohn, 2010). However, since considerable characterizations of the domestic landrace pig are applicable and valid for the minipig assessment, this strain will also be included in the evaluation (Forster et al., 2010).

4.2. Anatomical considerations

The size of the GIT regions, in relation to total body weight, in pigs is generally very similar to human. The stomach, small intestine and large intestine represent approximately 0.45/0.95% (landrace/Göttingen minipig), 2% and 1.4%, respectively, in pigs in comparison to 0.7%, 2.5% and 1.8%, respectively, in humans (Bollen et al., 1998; Kühn, 2001; Phuc and Hieu, 1993; Price et al., 2003). As in humans, the pig is monogastric and acid secretion results as a function of stimuli, such as food intake (Schubert, 2009; von Rosenvinge and Raufman, 2010). The length and diameter of the small intestine is 470–2000 cm (17–19 cm/kg) and 2.5–3.5 cm in landrace pigs and 832–900 cm (34–63 cm/kg) and 2 cm in minipigs (Bergman et al., 2009; Glodek and Oldigs, 1981; Kurihara-Bergstrom et al., 1986; Merchant et al., 2011; Suenderhauf and Parrott, 2013). In landrace pigs the large intestine (cecum:colon) is 23:413 cm (0.22:4 cm/kg) while in minipigs it is 20:303 (0.23:10 cm/kg), the colonic diameter is 2.7 cm (Glodek and Oldigs, 1981; McRorie et al., 1998; Merchant et al., 2011; Suenderhauf and Parrott, 2013).

Stomach fasting pH is highly variable (1.2–4.4) with indication of different pH regions within the stomach while the small intestine pH was reported to be about 7–8 (Hossain et al., 1990; Oberle and Das, 1994). No information of the pH in the large intestine could be found for a confirmed fasted state. In the fasted state the gastric transit time of nondisintegrating dosage forms was shown to be significantly retained (1–28 days) with high variability (Hossain et al., 1990). The transit times in small and large intestine were shown to be shorter and less variable (<1–3 days) (Hossain et al., 1990). In the fed state the gastric pH was reported to be 3–4.5 and the transit was generally shorter (1–6 h) than in the fasted state but still highly dependent on the gastric content (Merchant et al., 2011; Oberle and Das, 1994). The small and large intestine transit times in the fed state are less variable about 3–4 h and 24–48 h, respectively, and the pH ranges from 4.7 to 7.2 (Braude et al., 1976; Davis et al., 2001; Hossain et al., 1990;

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Ruckenbusch and Bueno, 1976; Wilfart et al., 2007). However, the transit time is somewhat influenced by intestinal content, for an example, a high fibre diet generated a value of 26 h while other liquids and solids had a transit time of 25–49 h (van Leeuwen et al., 2006; Wilfart et al., 2007). The water content, given as wet mass, for pigs fed *ad libitum* has been reported to be 250 g in the stomach and 500 g (0.25 g/cm gut length) and 750 g (1.7 g/cm gut length) in the small and large intestine, respectively (Merchant et al., 2011). Bile is stored in a gall bladder and secreted to duodenum as in humans. It is produced at a rate of approximately 20–50 μl/min/kg with a concentration of 42–55 mmol/L and has a similar composition to human bile (Bergman et al., 2009; Juste et al., 1983; Nakayama, 1969; Petri et al., 2006; Sjödin et al., 2008). The amplification of surface area due to folding and villi was found in landrace pig to be 3.7 in the small intestine and 2.5 in the large intestine (Snipes, 1997). Even though a systematic investigation or compilation of pig permeability data is absent, a number of single pass intestinal perfusion studies *in situ* have been completed where P_{eff} were determined. For some of these compounds, there are human P_{eff} reference values available, for instance P_{eff} (10^{-4} cm/s) pig:human: fexofenadine (0.02:0.07), verapamil (~1:6.8), antipyrine (0.61:5.6), cyclosporine (0.62:1.65) (Chiu et al., 2003; Persson et al., 2008; Petri et al., 2006; Thörn et al., 2009). For other APIs, a direct measurement of human P_{eff} is absent, such as in the case of danazol (pig: 1.1×10^{-4} cm/s) (Persson et al., 2008). Methodological aspects, e.g., surgery and anaesthesia, as well as species-related differences in physiology have been suggested to the lower P_{eff} values measured in pigs (Fagerholm et al., 1996; Petri et al., 2006). *In vitro* permeability investigation using pig material is limited. However, there are examples of studies with excised mucosal tissue using the Ussing chamber technique as well as employing isolated and cultured enterocytes (Bader et al., 2000; Nejdfors et al., 2000; Winckler et al., 1999).

4.3. GI metabolism

There are similarities between man and pig with regard to biotransformation, but also significant differences. E.g., pigs have very low CYP2D and CYP2C19 expression compared to man (van der Laan et al., 2010). In other cases, the metabolic activity in the intestinal wall is significant. This can be similar to human but in other cases not, e.g., the bioavailability of metoprolol is 48 ± 22% in humans compared to ~3% in pigs (Holm et al., 2013a; Sandberg et al., 1991; van der Laan et al., 2010). Not all human CYP isoforms have an ortholog in pigs (Helke and Swindle, 2013). Comparison between pig and human cDNA and amino acids show high sequence homology, though metabolic responses may vary as also demonstrated with the metoprolol example (Lu and Li, 2001; Monshouwer et al., 1998; Toutain et al., 2010). In Table 3, the CYP enzyme activity in landrace and Göttingen minipigs can be found and an overview of the CYP isoenzyme involved in the reactions with a number of substrates are presented in Table 4, demonstrating some differences in the CYP isoenzymes involved between the two species. A comprehensive characterization of abundances and substrate specificity of conjugating enzymes, e.g., UGT's and SULT's, is still absent for the pig. However, *in vitro* and *in vivo* studies have shown intestinal and hepatic UDP and SULT functionality with potential similarity to humans (Gu et al., 2006; Rahikainen et al., 2013; Sjögren et al., 2012; Thörn et al., 2012).

Altogether, based upon substrates, induces, inhibitors and regulation data, there are no major differences among CYP1A1, 1A2, 2B, 2E1 and 3A in pigs when compared to humans (Puccinelli et al., 2011). Hence, the pig could be a good model for human (with respect to metabolic profile) for compounds that are mainly metabolized by these CYP enzymes, However, as less is known of CYP2C, CYP2D6 and phase II metabolism in pigs, more caution should be taken when selecting pigs as a model for these compounds which interact with these enzymes.

4.4. GI transporters

Only a limited number of references on drug transporter expression and functionality in pigs and minipigs are available. However, Schrickx presented an RNA and protein expression study on the landrace pig of P-gp, BCRP and MRP2 in various tissues including the GIT (Schrickx, 2006). P-gp and BCRP RNA levels were found to increase along the small intestine to reach highest

Table 4
CYP test reactions and human and porcine isoenzymes involved.

Substrate	Human	Porcine	Reaction	Reference
Methoxyresorufin	CYP1A2	CYP1A	O-Demethylation	1
Ethoxyresorufin	CYP1A2	CYP1A	O-Demethylation	1–2
Coumarin	CYP2A6	CYP2A	7-Hydroxylation	2–6
Nicotine	CYP2A6	CYP2A/NI	O-Oxidation	7
Benzyloxyresorufin	CYP2B6	CYP2B? (NI)	O-Debenzylation	8
7-Ethoxy-4-triflouromethylcoumarin	CYP2B6	CYP2B? (NI)	O-Debenzylation	3
	CYP2B6	CYP2B? (NI)	Dealkylation	9, 10
Pentoxyresurfin	CYP2B6	ND	Demethylation	2
Mephenytoin				
Diclofenac	CYP2C8/9	CYP2C9/NI	4-Hydroxylation	3, 8.16
Tolbutamid	CYP2C9	NI	4-Hydroxylation	11
S-Mephenytoin	CYP2C19	ND	4-Hydroxylation	2, 3, 8
Desbrisoquine	CYP2D6	ND	4-Hydroxylation	12
Bufuralol	CYP2D6	CYP2B	1-Hydroxylation	3, 12
Dextromethorphan	CYP2D6	CYP2B	O-Demethylation	12, 16
Clorzoxazone	CYP2E1	CYP2E1+2A+3[a]	6-Hydroxylation	2, 3, 9, 13
p-nitrophenol	CYP2E1	CYP2E1 + CYP2A/NI	2-Hydroxylation	1, 14
Aniline	CYP2E1	CYP2A/(NI)	4-Hydroxylation	1
Midazolam	CYP3A4	CYP3A	1- and 4-hydroxylation	15
Testosterone	CYP3A4	CYP3A	6β-Hydroxylation	2, 3
Nifedipine	CYP3A4	CYP3A	N-oxidation	2, 9

NI: specific porcine CYP isoenzyme responsible for this reaction not yet identified ND: no detectable metabolism.
(1) (Nebbia et al., 2003), (2) (Skaanild and Friis, 1999), (3) (Bogaards et al., 2000), (4) (Skaanild and Friis, 2000), (5) (Shimada et al., 1994), (6) (Pelkonen et al., 2000), (7) (Skaanild and Friis, 2005), (8) (Myers et al., 2001), (9) (Desille et al., 1999), (10) (Behnia et al., 2000), (11) (Anzenbacher et al., 1998), (12) (Skaanild and Friis, 2002), (13) (Wiercinska and Squires, 2010), (14) (Skaanild and Friis, 2007), (15) (Lu and Li, 2001), (16) (Thörn et al., 2011).

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expression in distal jejunum with reduced expression in ileum and negligible amounts in the large intestine. The same expression pattern was observed for MRP2 with the difference that the expression was quite significant in the large intestine. This work also presented high homology between human and porcine P-gp with regard to transcriptional, protein and functional level. The work of Tang and co-workers reported an increased P-gp protein expression along the small intestine and also some expression in large intestine in the Yucatan minipig (Tang et al., 2004). Minor expression in the intestine (region unknown) has also been reported for a pig homolog to human OATP1A2 with high similarity both in sequence and functionality (Yu et al., 2013).

No *in vivo* study has been published on pigs with direct information of the intestinal transporters, e.g., PK data presented together with information of protein expression or genetic heterozygosity. However, several studies have been conducted using the domestic landrace pig with known substrates of transporters with or without transporter inhibitors, such as fexofenadine, ximelagatran, rosuvastatin, verapamil and digoxin (Bergman et al., 2009; Petri et al., 2006; Sjödin et al., 2008; Tannergren et al., 2006; Thörn et al., 2009). A few *ex vivo* studies wherein brush-border membrane of pig small intestine showing expression of carriers of amino acids and D-glucose have also been published (Munck et al., 1995) (Maenz and Patience, 1992; Munck et al., 2000; Scharrer et al., 2002; Stevens et al., 1982). As this section demonstrates, further investigations are needed to increase the knowledge and role of intestinal transporters in drug absorption in pigs.

4.5. Concluding remarks and gaps to be filled

The slow and variable gastric emptying of the pig is an important species difference to human with high potential implications for the *in vivo* GI drug absorption of enteric coated or modified release formulations as well as investigations in the fed state. In contrast to gastric transit times, the transit times in small and large intestine are generally less variable and more comparable to that in humans. Also, as pointed out by this summary, further characterization of pH and transit times in the fasted state is necessary, especially for small and large intestine and disintegrating formulations. Further investigations, both *in vitro* and *in vivo*, for the characterization of the intestinal permeability, metabolism (especially mediated by CYP2C, CYP2D6 and phase II enzymes) and transporters are also needed for an optimum application of the pig as a pre-clinical model for drug absorption. Anatomical and physiological data of the GI tract of pigs is summarized in Table 1.

5. Rat GI characterization

In general there are important differences between the physiology of rodents and humans such as the fact that rodents are nocturnal animals with consequences for timing of dose and an option to change day–night rhythm. Further rodents are prone to coprophagy with consequences of re-uptake of fecal excreted drugs. In addition, the size of rodents limits their use in studies with intact dosage forms intended for human use and generally higher metabolism is observed in rodents as compared to humans. Drugs are often dosed per body weight in animal studies and if comparing gastric volumes adjusted for body weight, the relative gastric volume of the rat is larger than for humans (Davies and Morris, 1993). The gastric emptying rate of liquids in the fasted state, being the most relevant factor when comparing drug absorption rates of BCS class I compounds, is somewhat similar in rats and humans, with a gastric emptying half-life around 15–30 min (Langguth et al., 1994). Gastric emptying in the rat is mainly controlled by the energy content of the ingested food in a similar manner like in humans. For instance, the gastric emptying half-life for solution with 0–6 kcal were 5–65 min (Maerz et al., 1994). The rat often has a higher gastric pH than that of man of about 4–5 (Davies and Morris, 1993; Kararli, 1995). Despite the increased gastric secretion, fasted gastric pH is generally increased by food intake across all species. The magnitude of gastric pH increase is highly dependent on the meal composition and a direct comparison between species is difficult. The contents of GI fluids such as bile salts, lipids and buffer species as well as the GI motility and pH are the main factors responsible for the initial saturation. The secretion of bile acids (taurocholate is the major bile acid at a total concentration of 8–25 mM in rats) is induced endogenously by food intake. In rats, within 10 min the secretion of bile-pancreatic juice proteins increased from 0.2 mg under fasted state with oral saline solution to 0.7 mg under fed state with oral fatty acids or sucrose solution (Hiraoka et al., 2003). Total bile acid and phospholipids concentration for the rat intestine is changing with segment such that saturation solubilities of compounds also depend on the segmental fluid composition and distribution (Tanaka et al., 2012).

The GI fluids mostly control the *in vivo* drug solubility, along with the volume of the co-administered water and the administered dose. The pH in small intestine increases continuously from duodenum to terminal ileum within a similar range as humans, i.e., 4.5–7.5. SITT of 3–4 h in rats is also similar to humans (Davis and Wilding, 2001; Lennernäs and Regårdh, 1993).

The anatomical features of the GI tract such as radius and length, microbial content, the hydrodynamic characteristics of volume, flow rate of the GI fluids, as well as elements closely associated with permeability for example tight junction have previously been reviewed (DeSesso and Jacobson, 2001; Kararli, 1995). Focusing on the epithelial permeability in small and large intestine, the rat has been used in several *in situ* single pass perfusion experiments (Cao et al., 2006; Fagerholm et al., 1996, 1997, 1999). This technique has, in several reports, shown that the rat jejunal permeability correlates strongly with the corresponding human jejunal P_{eff}. The P_{eff} for passively absorbed drugs on average was 3.6 times higher in humans compared to rats. Compounds with carrier-mediated absorption deviated from this relationship, which indicates that scaling of these processes needs further consideration (Cao et al., 2006; Fagerholm et al., 1996, 1999). Mechanistic investigations by determining human and rat jejunal permeability and at the same time examining the expression levels of transporters and metabolic enzymes through GeneChip techniques has found the same good correlation between human and rat permeability ($R^2 = 0.8$), only a moderate correlation for the transporter expression levels in duodenum ($R^2 > 0.56$), but no correlation in metabolizing enzyme levels (Cao et al., 2006). This agrees with the well-established difference between the species in drug metabolism and oral bioavailability where a mean allometric coefficient of 0.66 was determined in a correlation of plasma clearance of 54 extensively metabolized drugs between humans and rats (Chiou and Barve, 1998). On the basis of these studies, the rat *in situ* single-pass perfusion model is considered as the most appropriate animal model for predicting human permeability and absorption from the small intestine (Cao et al., 2006; Fagerholm et al., 1996, 1997, 1999). In addition, the Ussing chamber with rat tissue has also been reported to be a useful model to predict human intestinal absorption based on data from both the small and large intestine (Lennernäs et al., 1997; Ungell et al., 1998). It seems that the rat colon may be useful to predict absorption of drugs intended to be used in oral MR dosage forms but this requires further validation. An opportunity can be seen in the assessment of absorption

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for BCS II, III and IV drugs in IR dosage forms using this technique because of the regional differences. However, even if the rat is a suitable preclinical model for GI absorption it has limitations such as body size, dietary intake difference and the shortcomings related to dosing of intact solid dosage forms.

6. Mouse GI characterization

6.1. Anatomical considerations

Although a description of the anatomy and physiology of the mouse GI tract can be found in various previously published reviews (de Zwart, 1999; Kararli, 1995; McConnell et al., 2008a; Ogiolda et al., 1998), the amount and quality of the data regarding biopharmaceutically relevant information in mice in particular for drug product development is much less than human and rat, although the mouse has been the most extensively studied species in pharmacology.

The mouse stomach is divided into a glandular and non-glandular portion (de Zwart, 1999). The non-glandular portion is thin--walled and is a higher percentage than found in humans. The glandular portion is thick-walled and secretes mucus, pepsinogen, and HCl. Ogiolda studied the size of various parts of the mouse GIT at 8-months of age after selective breeding of 8-week old mice (Ogiolda et al., 1998). The body weights at 8-months of age resulted in the following categories: heavy (63 g male, 58 g female), light (22 g male, 21 g female), and randomly selected (45 g male, 41 g female). Since most of the anatomical and physiological publications relate to mice that are 18–22 g we will focus on the average properties of male and female mice reported by Ogiolda for the "light" category. McConnell reported that a 20 g mouse is comfortably full with a stomach volume of 0.37 mL and could expand up to 0.71 mL maximum (McConnell et al., 2008a). Assuming the stomach is a sphere with surface area of 2.47 cm^2, the radius would be 0.443 cm and the resulting spherical volume would be 0.365 mL. Thus, there is a good correlation between the data collected by Ogiolda for the "light" category mouse and the physiological data of McConnell. More importantly, for estimation of mouse anatomy for a given body weight, Ogiolda did not find statistically significant differences between the "light", "random", or "heavy" mice when the ratios of GI section weight/body weight, or surface area/body $weight^{(2/3)}$, or length/body $weight^{(1/3)}$ were compared. Ogiolda found the following additional average dimensions for the "light" category mouse: Duodenum weight (166 mg) and length (38 mm); Jejunum/Ileum weight (1156 mg) and length (364 mm); Cecum weight (305 mg) and length (31 mm); and Colon weight (550 mg) and length (83 mm).

Assuming consistency and reported relationship to body weight of these GI parts, the regional anatomy for any size mouse should be easily estimated. In fact, these dimensions are consistent with Kararli who reported a total SI length of 35–45 cm for mice (Kararli, 1995). In a study of the influence of lactation on processing of dietary protein, Harmatz et al. reported that the average circumfence of the control mouse (21 g) small intestine was 0.7 cm (Harmatz et al., 1993). The radius determined from this value of circumference would be 0.11 cm and if it is assumed that the small intestine is a cylinder of 36 cm long, its volume would be 1.38 mL. Of course, the small intestine does not have a constant radius going from the duodenum to the distal ileum, but more accurate measurements of the radius for different segments of the mouse intestine are not available. McConnell et al. measured the average water content of the mouse small intestine (70%) by weighing the contents before and after lyophilization and estimated fasted water volume to be 0.81 mL and fed water volume to be 0.98 mL. Thus, there is very good agreement of total volumes and water volumes for the mouse small intestine from a variety of studies by different groups over more than a decade.

6.2. Gastric emptying and small intestinal transit time

Recently, technetium-labeled activated charcoal diethylenetriaminepentaacetic acid (99mTc-Ch-DTPA) detected by single-photon emission computed tomography (SPECT) was used to study gastrointestinal transit times in mice (Padmanabhan et al., 2013). It was found that stomach transit time was 1 h, small intestinal transit time was 1–20 h, and cecum and colon transit time was 3 h. In a study of the influence of salmon calcitonin on the small intestinal transit time (SIT) in the mouse, Hamada et al. reported the average non-inhibited SIT to be 1 h (Hamada et al., 1999).

6.3. pH of the mouse small intestine

McConnell et al. investigated the pH, water content, and lymphoid tissue distribution in two groups of mice (McConnell et al., 2008a). The first group was fasted overnight with free access to water and the second group was given free access to a low protein (18%)/low fat (5%) standard diet and water at all times. When 9.17 g of mouse chow was mixed with 10 mL of water until the food pellet disintegrated, the resulting pH was 5.9. GI pH was determined after sacrificing the animal, by dissecting the GI segments, collecting and mixing the undiluted contents, and measuring pH with a meter. In contrast to humans, mice had lower stomach pH (3.0) under fed conditions than fasted (4.0 ± 0.3). Under fasting conditions, all sections of the mouse small intestine had similar pH values and were slightly higher (5.0 ± 0.25) than in fed mice (4.8 ± 0.03). Compared to the fasted human small intestine where the pH ranges from 6 to 7.4, the lower pH in the mouse has implications for *in vivo* testing. When delivered as a solution formulation, acidic drugs may precipitate in the mouse small intestine but remain in solution or dissolve readily when tested in humans. Also, when tested in mice, the performance of pH-sensitive polymeric delivery formulations may not exhibit the same release characteristics as expected in humans. In contrast to the differences in normal values of pH between human and mouse, in a study of the intestinal permeabilities of the following five model drugs furosemide, piroxicam, naproxen, ranitidine and amoxicillin in the *in situ* intestinal perfusion technique in mice, it was found that permeability was similar for the above mentioned compounds when compared to human (Escribano et al., 2012).

6.4. Metabolism and transport

Information on the quantitative expression of intestinal metabolic enzymes in the mouse is not as plentiful as some other preclinical species. Zhang et al. reported on mRNA expression and identity of the following Cytochrome P450 enzymes; Cyp1a1, 1b1, 2b10, 2b19, 2b20, 2c29, 2c38, 2c40, 2e1, 3a11, 3a13, 3a16, 3a25, and 3a44 (Zhang et al., 2003). They found that Cyp3A13 was found in the highest abundance in the small intestine followed by 3a11. However, liver expression of 3a11 was higher than 3a13. Komura et al. published on species differences for *in vitro* and *in vivo* small intestinal metabolism for several CYP and UGT substrates in mice (Komura and Iwaki, 2008, 2011). Mutch et al. commented on regional variability in ABC transporter expression in mice but less information is available for other drug transporters (Mutch et al., 2004). Finally, Holmstock et al. has reported on a transgenic mouse model that has human CYP3A4 and P-gp for use in studying the inducing effects of xenobiotics on human intestinal P-gp (Holmstock et al., 2013).

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6.5. Concluding remarks and gaps to be filled

The mouse GIT shows some similarity to human and both species share the same finger-shaped morphology of intestinal villi. This is in contrast to rats with tongue-shaped villi (de Zwart, 1999). However, the lower pH of the small intestine might make the mouse less attractive for formulation development than some of the other preclinical species. More quantitative information on intestinal enzyme and transporter expression will facilitate the use of physiologically based pharmacokinetic (PBPK) models for *in vitro/in vivo* extrapolation.

An overall comparison of anatomical and physiological data of the GI tract of humans and commonly used animal models is given in Table 1.

7. Imaging technologies for anatomy, physiology and, dosage form performance

Imaging has proved to be a useful tool in biopharmaceutical studies as it enables a number of important attributes related to the formulation. In particular, the following information can be acquired:

- Patterns of motility and the transit of material along the GIT.
- The region in which dispersion/disintegration occurs.
- Time point at which release of material occurs.
- The influence of feeding on dosage regimens: effects of dosing relative to a meal; influence of food components.
- The amount of water available for dissolution.
- The separation of meal components.
- Gall bladder volumes.
- *In vivo* erosion and release rates of MR systems and enteric coatings.

When combined with other measurements, the imaging techniques become even more informative as the combination of data can be used for additional elucidation of aspects such as:

- Regional absorption (e.g. extent of colonic absorption).
- Localization of the dosage form in the GIT relative to the plasma concentration (sometimes termed 'pharmacoscintigraphy' or 'pharmacomagnetography').
- The unambiguous relationship between position in the GIT and pH for tagged radiotelemetry devices.

The use of imaging has been reviewed, sometimes at length, in several articles over the past 30 years. These articles have dealt with specific technologies: gamma scintigraphy (Hardy and Wilson, 1981; Newman and Wilding, 1999; Wilding et al., 2001) MRI (Marciani, 2011; Schwizer et al., 2006) and magnetic marker monitoring (MMM) (Weitschies et al., 2010, 2005). MMM is also referred as magnetic moment imaging (MMI), magnetic pill tracking as well as related methods such as alternating current biosusceptometry (ACB) (Cora et al., 2011). Characteristics of the currently most used imaging modalities have recently been reviewed (Weitschies and Wilson, 2011). In most cases a separate marker compound is used in a formulation (e.g. with drill & fill method), but this may have a different release/dissolution profile than the API from the non-modified formulation.

A very promising recent development is the MR-based combination of ^{19}F tracking and ^{1}H imaging allowing real time tracking of one or more ^{19}F labeled dosage forms (Hahn et al., 2011, 2013). In addition, the availability of new telemetric tools for clinical gastroenterology such as the Given Imaging camera pill (Glukhovsky and Jacob, 2004) and those for investigational studies including the Medimetrics IntelliCap pH sampling unit (Shimizu et al., 2008) and the SmartPill pressure sensor (Cassilly et al., 2008), have been applied in biopharmaceutic studies. Specific details concerning these instruments can be found in engineering journals (McCaffrey et al., 2008). Optical systems have been used to examine deposition in the lower gut and in the vagina (Henderson et al., 2007). This technology is best applied to fluorescent labels or materials that have intrinsic fluorescence, aiding biopsy sampling (Muldoon et al., 2007). The IntelliCap system is in principle also capable of sampling GI fluids, the issue being preservation of the integrity of the sample.

7.1. MRI in biopharmaceutics

The understanding of functions of the GIT is important in the application of biopharmaceutics for the development of orally administered drugs. Experimental approaches have mainly relied on tissue sections to examine the microscopic structures within the GIT as well as telemetric measurements to understand transit time, gastric emptying time and pH changes along the length of the GIT in humans and animals. While understanding human physiology is of the utmost importance to the development of new orally acting drugs, the ability to make comparisons across typical species used in drug development, such as the rat, dog and pig, are useful in extrapolating from these animal species to humans. In fact any of the measurements in current *in silico* tools for these extrapolations have embedded within them the characteristics of these animal species.

Imaging techniques have been used frequently to understand how specific compounds (new chemical entities) or probe compounds interact with the various components of the GIT through measurement of compound in blood, plasma or urine into which the compounds find their way. The compounds are typically labeled with radioactive functional atoms which can be measured either based on gamma emissions (scintigraphy, e.g., Single Photon Emission Tomography, or positron emission tomography) (de Kemp et al., 2010; Shoghi, 2009; Van Berkel et al., 2008). In these two cases, the radioactive nuclei, ^{99m}Tc or ^{18}F, are short-lived isotopes which decay rapidly with half-lives in the order of 6 h and 110 min, respectively. Moreover, newer techniques have been developed to measure tissue concentrations in thin sections (e.g., matrix-assisted laser desorption ionization, MALDI); however, as with the other techniques they measure compound and metabolites and do not focus on microscopic or patho-physiological processes.

Functional imaging on the small animal level has not been used frequently to date but has been applied for many years to humans. The clinical MRI equipment is quite expensive and difficult to justify for use in small animals. Approximately 25 years ago, small animal MRI instruments were developed and recently increased efforts have been made in the field (Hockings, 2006; Rudin, 2005).

Several obstacles limit MRI technologies in pharmaceutical preclinical animal research: animal size called for adapting instrument dimensions and magnetic field strength; the necessity for anesthesia and its monitoring, the control of body temperature among other vital parameters, all these requirements have to be met for a successful longitudinal, repetitive and non-destructive application of MRI.

From a scientific point of view, the advantages of such non-invasive technologies include the ability to work with intact animal models, much closer to human reality than any isolated cell suspension system, in spite of the tremendous difficulties often encountered. The improved predictivity of (patho)-physiological data obtained in living animal models as a basis for subsequent human studies responds to the ethical requirement of utmost safety before human application.

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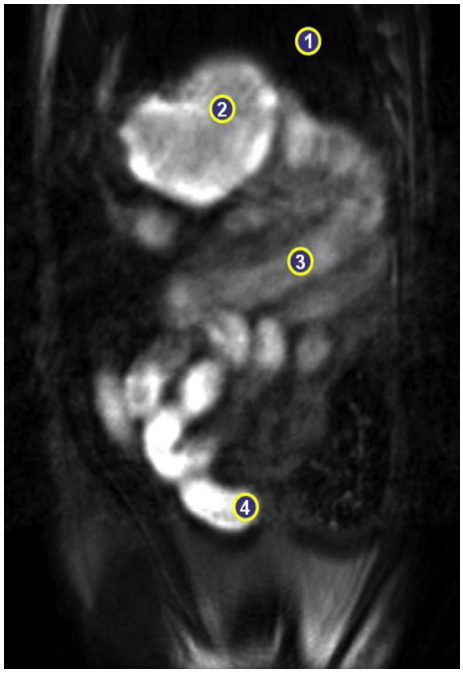

Fig. 4. MRI of rat abdomen. To improve delineation of the intestine a mannitol solution was applied per gavage. Numbers indicate organs as follows: (1) Liver, (2) Stomach, (3) Small intestine, (4) Colon. MRI details: Bruker BioSpin 70/30: T2-weighted RARE-image, TR 2000 ms, MTX 256, FOV 8 × 6 cm; toal acquisition time, 8 m 32 s. Image kindly provided by Sanofi-Aventis.

In vivo MRI as a tool for animal research is very much in line with the Ethics Committee recommendations of decreasing animal use since, usually, the chosen MRI-parameter acts as a biomarker for the interesting physiological process or function of interest and is determined dynamically over a suitable period of time. Therefore, it is not only a snap shot of this process, but depicts the development up to its final "end-point" with an inherently higher reliability.

MRI in drug development has mainly addressed the understanding of normal physiological versus pathological activity of, for example, the heart or other organs and the effects produced by drugs on that tissue. Rarely has MRI technology been used to understand the effects of excipients on gastric motility or formulation effects in the GIT of small animals in order to better understand the human situation.

Contrast agents are tolerated by small animals and similar to humans, they can be used to look into the physiology of the GIT (Fig. 4). The work proposed in OrBiTo is to develop best practices for the most commonly used species, rat and Beagle dog using MRI to investigate function, water content, mucus layers (Fig. 5) and motility in these species. Assessing how these factors correlate to man with regard to formulation disintegration and dissolution, particle and macromolecule (e.g. excipients) diffusion through the unstirred water layer (i.e., the mucus compartment) and its final distribution and disposition will aid in selecting the best model, reduce the number of animals needed to optimize a formulation and provide input to model builders in the other work streams.

8. GI luminal concentration profiling of orally administered drugs in humans

GI drug concentrations after oral administration reflect the interplay of multiple GI and biopharmaceutical processes, including the drug dose, drug release, dissolution, solubility, transit and simultaneous dilution by secretions, precipitation, degradation and mucosal permeation. Monitoring these luminal concentrations as a function of time, therefore, provides a unique insight into the intraluminal behavior of a drug and its formulation (Fig. 6).

8.1. Methodology: sampling and characterizing GI fluids

The determinations of the GI drug concentrations can be performed following oral administration to healthy volunteers, where GI fluids are sampled by means of a double-lumen catheter, positioned either via the mouth or nose into the upper GIT. Intubation with two catheters allows the simultaneous assessment of drug concentrations at two intraluminal positions: e.g., the stomach and duodenum (Brouwers et al., 2007b; Walravens et al., 2011) or duodenum and upper jejunum (Brouwers et al., 2005, 2006). Positioning of the catheters is usually monitored by means of fluoroscopy (Lennernäs et al., 1992). Drugs are administered either orally (dosage form with water) (Brouwers et al., 2005, 2007b, 2006; Walravens et al., 2011) or directly into the stomach by means of a catheter (solution or suspension) (Psachoulias et al., 2011; Vertzoni et al., 2012). While 'real-life' administration is obviously most relevant, gastric dosing may be more appropriate for mechanistic purposes. To this point, Psachoulias et al. investigated intestinal precipitation of weakly basic drugs upon gastric emptying by administering drug solutions directly into the stomach; this approach neglects gastric dissolution to focus on supersaturation and precipitation (Psachoulias et al., 2011). Fed state conditions can be simulated by either a nutritional drink (Brouwers et al., 2007b) or a homogenized liquid meal (Vertzoni et al., 2012).

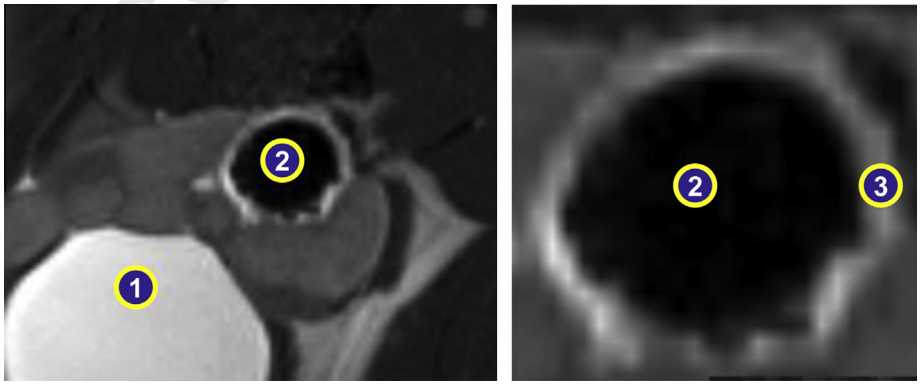

Fig. 5. MRI of rat intestinal mucosa. High signal intensity rim in colon, tentatively assigned to mucus layer. Mucus thickness approximately 700 μm, requires further validation. Numbers indicate regions as follows: (1) bladder, (2) Colon lumen, (3) Rim of mucosa. The image to the right is a magnification of the region of the colon. MRI details: Bruker BioSpin 70/30: T2-weighted RARE-image, TR 2000 ms, MTX 256, FOV 7 cm; total acquisition time, 2 min. Image kindly provided by Sanofi-Aventis.

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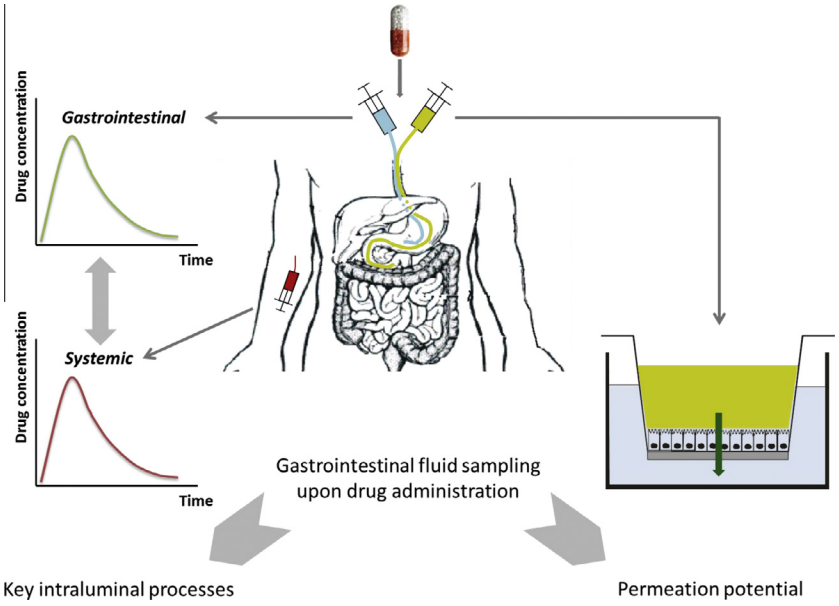

Fig. 6. Gastrointestinal fluid sampling upon drug administration in humans as a unique approach to identify intraluminal processes that are key to the overall drug absorption process and the investigation of the mucosal permeation potential of a drug.

GI fluids are sampled as a function of time, typically for a period of 2–4 h following administration. To minimize the effect of sampling drug on the absorption process, aspirated volumes should be kept to a minimum, especially when multiple GI sites are sampled and/or plasma concentrations are determined in parallel. At the time of sampling, enzymatic processes should be inhibited (e.g. cocktail of lipase and protease inhibitors), non-dissolved and/or precipitated drug particles should be separated (centrifugation or filtration) and possible further precipitation should be avoided by diluting the particle-free sample. The observed drug concentrations will be related to characteristics of the GI fluids such as: pH, osmolality, viscosity, surface tension, buffer capacity and concentration of bile salts, phospholipids and dietary (digestion) products.

8.2. Applications of GI concentration profiling in humans

8.2.1. Fosamprenavir: understanding an unexpected food effect

Fosamprenavir is a phosphate ester prodrug of the poorly soluble HIV protease inhibitor amprenavir. To investigate *in vivo* the intraluminal dephosphorylation of fosamprenavir, required for transepithelial permeation, GI fluids and plasma samples were collected after administration of an IR tablet of fosamprenavir (Telzir®) to healthy volunteers in the fasted and fed state (Brouwers et al., 2007b). The plasma concentrations of amprenavir demonstrated a distinct food-induced delay in absorption (mean t_{max} increased by 2.5 h). Previous *in vitro* studies suggested that the inhibition of fosamprenavir dephosphorylation in fed state conditions as a potential cause of this delay (Brouwers et al., 2007a). However, the clinical study revealed duodenal appearance of fosamprenavir, and not dephosphorylation, as the major determinant of plasma t_{max}. In the fed state, duodenal appearance appeared to be delayed due to an unexpected delay in gastric disintegration of the IR tablet of fosamprenavir. This study clearly illustrated the use of GI concentration profiling to identify key intraluminal processes for absorption. In addition, the data were used as reference to validate the TNO Intestinal Model (TIM) to predict food-dependent disintegration of IR tablets (Brouwers et al., 2011).

8.2.2. Gastric dissolution of weakly basic drugs and the role of acidic beverages

Posaconazole is a poorly soluble and weakly basic antifungal drug, administered as an oral suspension (Noxafil®). GI concentration profiling in healthy volunteers confirmed the importance of gastric dissolution for posaconazole absorption (Walravens et al., 2011). In line with other weakly basic drugs, fasted state intake of posaconazole with an acidic cola beverage significantly enhanced absorption by improving gastric dissolution. In contrast to the usually accepted hypothesis, this could not necessarily be attributed to a reduction of the gastric pH. In normal fasted state conditions, the acidic beverage enhanced dissolution by prolonging the gastric residence time without affecting the gastric pH.

8.2.3. The role of intestinal precipitation in the absorption of weakly basic drugs

Intraluminal drug precipitation from supersaturated solutions is currently extremely difficult to predict, since the influence of GI physiology on the precipitation process has not yet been elucidated. By characterizing intestinal fluids aspirated after gastric administration of solutions of the weakly basic drugs ketoconazole and dipyridamole, intestinal precipitation was recently investigated for the first time *in vivo* (Psachoulias et al., 2011). As expected, the intestinal fluids appeared supersaturated with both drugs as a result of the solubility difference between stomach and intestine. In contrast to previous *in vitro* experiments (Kostewicz et al., 2004), however, the observed precipitation *in vivo* was minimal. The obtained clinical data were used to optimize the experimental conditions of *in vitro* transfer experiments for weak bases, pursuing more accurate and biorelevant prediction of intestinal precipitation (Psachoulias et al., 2012).

8.2.4. GI fluids as reference for biorelevant flux assessment

The above mentioned examples illustrate the unique value of GI concentration–time profiles to elucidate intraluminal processes crucial for absorption. Another application involves the use of aspirated GI fluids as a reference for the biorelevant *in vitro* assessment of drug flux across an epithelial monolayer. Flux is an informative measure of drug and formulation performance, since it not only

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depends on intraluminal drug concentrations but also on the permeability of the monolayer for the drug. Absorption-enabling strategies, including food- or excipient-based solubilization of lipophilic drugs, often generate highly complex intraluminal fluids that may drastically affect the permeation potential of the drug. Simulating these effects *in vitro* may be challenging and requires reference fluids. For instance, upon administration of a co-solvent and surfactant-based formulation of amprenavir (Agenerase®), intestinal fluids contained high amprenavir concentrations as a result of solubilization by D-α-tocopheryl polyethyleneglycol 1000 succinate (TPGS) (Brouwers et al., 2006). Subsequent use of these fluids as the donor medium in Caco-2 transport experiments demonstrated the interplay of multiple factors in determining the amprenavir flux: (1) increased amprenavir concentrations, (2) entrapment of amprenavir in TPGS-based micelles (reduced permeability), and (3) inhibition of the efflux carrier, P-gp, by bile salts and TPGS.

Similarly, intestinal fluids were collected and analyzed upon administration of the highly lipophilic drug danazol together with a heterogeneous liquid meal (Vertzoni et al., 2012). In comparison to a simple aqueous medium, diluted aspirates and micellar phases of aspirates significantly reduced the permeability for danazol across Caco-2 monolayers as a result of entrapment in the luminal coarse and/or micellar lipid structures. In terms of flux, however, the increased danazol concentrations in lipid structures overcompensated for the reduced permeability.

8.3. Future of GI concentration profiling

In comparison with the use of classic PK studies that require deconvolution or modeling to simulate drug appearance in the systemic circulation from other disposition processes, the sampling and analysis of GI fluids upon drug administration directly reflect intraluminal drug and formulation behavior. As such, the technique provides unique reference data for optimization of *in vitro* and computational models to assess drug absorption. Especially for drugs with a suboptimal absorption potential (BCS class II–IV) that rely on complex absorption-enabling strategies, e.g., solubilization and supersaturation, the predictive power of existing simulation models is insufficient. It can be expected that direct assessment of intraluminal drug concentrations will play a crucial role to resolve the performance of these strategies in the complex GI environment and to guide further optimization of models.

8.4. Regional absorption methodologies/in vivo and ex vivo animal models for characterizing segmental/regional drug absorption

The physicochemical properties of the API and the complex physiological and biochemical interactions of the GIT determine the regional intestinal P_{eff} *in vivo*. Variations in mucosa physiology may affect regional P_{eff} differently depending on the transport mechanism(s) involved (Chadwick et al., 1977a,b; Corrigan, 1997; Davis and Wilding, 2001; Lennernäs, 1998; Ungell et al., 1998; Winiwarter et al., 2003, 1998). Intestinal P_{eff} depends on the coexistence of multiple, parallel transport processes such as passive transcellular diffusion and carrier-mediated absorption and carrier-mediated efflux (Sugano et al., 2010).

8.4.1. Human model

Direct measurements of intestinal P_{eff} and secretion of drugs in humans are possible by regional intestinal perfusion techniques (Drescher et al., 2003; Igel et al., 2007; Lennernäs, 1998; Lennernäs et al., 1992, 1994; Modigliani et al., 1973a,b; von Richter et al., 2001). In general, four different clinical perfusion principles have been employed in the human small intestine (Fig. 7):

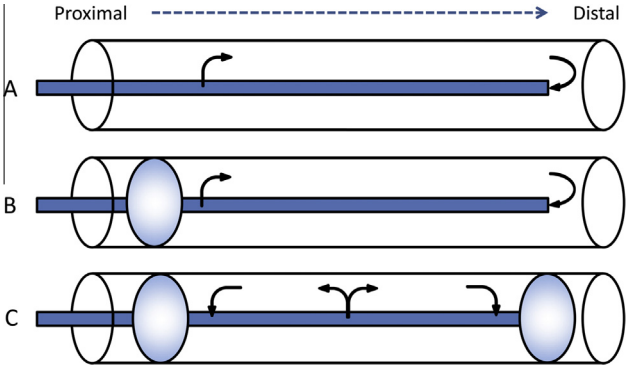

Fig. 7. Schematic illustration of experimental techniques for human intestinal perfusion studies. (A) Open perfusion system. (B) Proximal balloon perfusion system, (C) Double balloon perfusion system. Solid arrows indicate where the perfusate enters and leaves the intestinal segment. Generally the perfusate leaves the segment by force of gravity, as the fluid is collected on ice standing on the floor while the subject is positioned in a bed. The dotted arrow specifies the proximal-to-distal direction of the intestine.

- a triple-lumen oro-nasal tube including a mixing segment,
- a multi-lumen tube with a proximal occluding balloon,
- a multi-lumen tube (Loc-I-Gut®) with two balloons occluding a 10 cm long intestinal segment (Fig. 7),
- two 20 cm adjacent jejunal segments were isolated with the multi-lumen perfusion catheter.

The advantages and disadvantages of the various intestinal perfusion techniques are discussed elsewhere (Lennernäs, 1998). Direct determination of compound transport and metabolism through blood concentration measurements in the mesenteric and portal vein is not possible in humans for obvious reasons. Intestinal perfusion techniques based on drug disappearance and appearance in the lumen do, however, offer great possibilities of measuring various intestinal transport processes. Over the past 70 years different *in vivo* intestinal perfusion techniques have been developed and the importance of such *in vivo* work has been clearly demonstrated (Drescher et al., 2003; Igel et al., 2007; Lennernäs, 1998; Lennernäs et al., 1992, 1994; Modigliani et al., 1973a,b; Pfeiffer et al., 1990; Rambaud et al., 1973; von Richter et al., 2001). A good correlation between *in vivo* determined P_{eff} and historical data on fraction of dose absorbed (f_{abs}) for a large number of structurally diverse drugs have been established and reported (Lennernäs, 2007). The effects of the tube on GI physiology are minimal and do not question the pharmaceutical relevance of drug absorption data collected using these perfusion methods. For instance, Näslund et al. showed that there was no difference in the sensitive gastric emptying process between the following three methods: scintigraphic, oral dosing of paracetamol tracer and subsequent plasma sampling, and polyethylene glycol (PEG) dilution methods using intubation tubes (Näslund et al., 2000).

Validated double balloons methods have been used for regional single-pass perfusions of the proximal jejunum and distal rectum *in vivo* in humans on separate occasions. The small intestinal tube has been extensively used to examine jejunal P_{eff} of various compounds (Lennernäs, 1998; Lennernäs et al., 1992, 1994; Tannergren et al., 2003a,b). The jejunum is the major absorbing region for drugs and nutrients in most mammals. It also has the largest surface area and is the site of the most active carrier-mediated transport in the gut (Chadwick et al., 1977a,b; Collett et al., 1997; Hilgendorf et al., 2007; Lennernäs et al., 1992; Ungell et al., 1998). Human *in vivo* jejunal P_{eff} values for 42 compounds (31 drugs) have been determined using this technique and will be referred to later on (Chiu et al., 2003; Fagerholm et al., 1995,

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1996, 1997, 1999; Lennernäs, 1997, 1998; Lennernäs et al., 1992, 1994, 2002, 1993, 1997; Lindahl et al., 1996; Petri et al., 2006, 2003; Sandström et al., 1998, 1999; Sun et al., 2002; Söderholm et al., 1997; Takamatsu et al., 2001, 1997; Tannergren et al., 2004, 2003a, 2003b; Winiwarter et al., 2003, 1998).

The human *in vivo* jejunal P_{eff} (at pH 6.5) for fexofenadine, furosemide, hydrochlortiazide and inogatran is 0.07×10^{-4}, 0.05×10^{-4}, 0.04×10^{-4}, and 0.03×10^{-4} cm/s, respectively (Fagerholm et al., 1997; Tannergren et al., 2003a; Winiwarter et al., 1998). The f_{abs} for each drug is 30–41%, 40–60%, 55% and 5–10%, respectively. These drugs have hydrophilic properties and their passive transcellular diffusion is expected to be low. Also, they are too large to be considered to have a significant paracellular uptake, which is indicated by the low and incomplete f_{abs}. A potential explanation for the relatively high interindividual variability seen in the permeability estimates is that there is a small difference between inlet and outlet concentration in the perfusate. For the two diuretics, there was also a strong induction of fluid flux into the segment that might have affected the determination of the P_{eff} value.

Some human permeability data have been reported from studies in which a triple lumen tube was used to perfuse 80 cm segments in jejunum and ileum at a perfusion rate of 5 mL/min (Sutcliffe et al., 1988). From these data, it can be speculated that the P_{eff} values of these low permeability compounds are somewhat higher than that measured by the regional perfusion method with two balloons. This apparent discrepancy may be explained by pH differences and/or by the open nature of the triple lumen perfusion technique, which may have allowed absorption from a much longer segment than it actually was designed for (due to uncontrolled flow of perfusate in both directions). It is obvious that more exploratory *in vivo* studies are required in order to obtain reliable data on regional intestinal drug absorption. It is crucial to accurately determine the regional intestinal P_{eff}, as this information will contribute to form the basis for the expected increase in *in silico* predictions of oral biopharmaceutics. It is suggested that it would be feasible to use open, single-pass perfusion studies for the *in vivo* estimation of regional intestinal P_{eff}, but that care should be taken in the study design to optimize the absorption conditions.

8.4.1.1. In vivo animal models for characterizing segmental/regional drug absorption. A MR dosage form administered after fasting conditions reach the colon in most instances within 3–6 h (Follonier and Doelker, 1992). Thus, if longer duration of drug release and absorption is desired, drug absorption in colon is a prerequisite. In addition, for compounds with incomplete absorption in the small intestine, e.g., BCS class III, IV and certain class II drugs, some absorption may also occur in the more distal intestine for IR formulations. However, many drugs have too low and highly variable absorption in the colon to be suitable for extended release delivery (Wilding and Prior, 2003). In the worst case, poor colonic drug absorption may terminate the development of the MR product. Hence, a regional drug absorption assessed, preferable in humans, should be conducted prior to starting the MR formulation development. However, for new chemical entities it is desirable to evaluate regional absorption properties already in pre-clinical screening.

There has been a clear progress in recent years to use *in vitro* tools for regional absorption assessment including permeability aspects (Sjöberg et al., 2013; Tannergren et al., 2009), solubility aspects (Vertzoni et al., 2010), and drug degradation in colonic lumen by bacteria (Sousa et al., 2008). However, the advantage of *in vivo* models compared to *in vitro* testing is that they capture the complexity and dynamics of critical factors in the GI tract influencing drug release and absorption. The main limitation of the usage of animal models is that no single species resembles all physiological properties of man, even if the rat model seems to be predictive (Ungell et al., 1998). This introduces a risk that the results obtained in the animal model are not fully predictive for the situation in man. The main aspects to consider in deciding on using an animal model are physiological features of the different regions of the GI tract, such as surface area, tight junction pore size, intestinal transporters, residence times in different segments, physical and physicochemical characteristics and volumes of GI fluids, the presence of enzymes that could metabolize drugs and gut wall metabolism since these factors could directly affect regional absorption.

The dog is in many cases an acceptable model due to its similarity to man regarding anatomy, motility pattern, residence times and many secretory aspects (Dressman and Yamada, 1991; see also canine section in this review). This is further verified by the compilation of GI physiology data provided in Table 1. In addition, the size of dogs also allows for subsequent studies of formulations developed for human use. The focus of the current work is to review comparisons between man and dog regarding regional absorption allowing some general conclusions about suitability and role of the dog model. In addition, the use of rat for prediction of regional human drug absorption will also be briefly addressed.

8.4.2. Dog model

Several, different methods have been applied for regional absorption studies in dogs including endoscopic or colonoscopic methods (Sutton et al., 2006; Tajiri et al., 2010b), remote control capsules (Ishibashi et al., 1999b; Parr et al., 1999) and direct access to the intestine through surgical access ports (Kim et al., 1994).

The most extensively published comparison between dog and man covering the relative bioavailabilities following administration in more distal parts of the GI tract, primarily the colon, versus administration to the proximal small intestine has been performed by Sutton et al. (Fig. 8) (Sutton et al., 2006). Their study included 11 compounds and showed a good correlation ($r^2 = 0.8$) between dog and man regarding colonic relative bioavailability. In addition, the relationship of relative colonic bioavailability between dog and man was close to 1:1.

The study included drugs from all BCS classes. It was clear that low permeability compounds consistently provided low relative colonic bioavailability in both dog and man. Regarding low solubility compounds with high permeability (Class II), two out of three compounds had relatively high colonic drug absorption in both species. The greatest individual difference between dog and man was obtained for atenolol which had 67% relative colonic bioavailability in dog and only 15% in man. A similar investigation was performed within AstraZeneca using dogs with colonic fistulas for direct administration to this site where a corresponding correlation of $r^2 = 0.6$ was achieved. However, high permeability

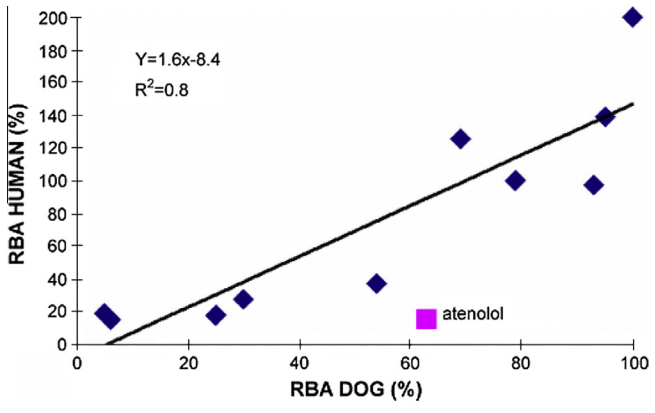

Fig. 8. Relationship between relative bioavailability in dogs and humans following oral and colon administration (Sutton et al., 2006).

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compounds had consistently higher relative bioavailability and low permeability compounds had lower values in agreement with the work by Sutton (Sutton et al., 2006). A couple of low permeability compounds, cimetidine and ranitidine, had significantly lower values in man compared to the dog as was noticed for atenolol (Sutton et al., 2006). This supports the suggestion by Sutton that for smaller hydrophilic molecules, the dog colonic mucosa is somewhat more "leaky" than in humans. Another interesting deviation between dog and man in the AstraZeneca data set was for an internal developmental compound where relative colonic bioavailability was 48% and 100% in dog and man, respectively. This compound was identified to have intestinal Phase II metabolism indicating that the regional difference with less metabolism in colon in man is not reflected by the dog model. Tajiri and co-workers studied diclofenac, dilitiazem, cevimeline, felodipine, morphine, metformin and felodipine in a dog colonic absorption model (Tajiri et al., 2010a,b). Again a good correspondence was obtained with human data where good absorption was obtained in man for all compounds except metformin. The main deviation was obtained for felodipine which is almost completely absorbed from long acting extended release formulations in man while colonic relative bioavailability was only 30%. This may be due to differences in *in vivo* dissolution between dog and man since felodipine is a low solubility compound (water solubility 1 μg/mL). However, an alternative or complimentary explanation is the difference between the species of regional intestinal CYP3A4 metabolism. The latter explanation is also supported by other data from Tajiri for diltiazem, where the relative colonic bioavailability in dog was lower than for man at relevant doses (Tajiri et al., 2010b). Also data from Sutton for nifedipine, another drug with intestinal CYP3A4 metabolism, showed lower relative colonic bioavailability in the dog compared to man, further supporting the possibility of species differences in regional intestinal metabolism. Recent work on gene expression of enzymes and transporters in beagle dogs also concluded that there are significant differences in distribution of CYP enzymes between dog and man (Haller et al., 2012).

In conclusion, the dog model seems relevant with respect to identifying drugs with permeability-limited colonic drug absorption even though absolute levels for some drugs in this class seem to be somewhat higher in the dog compared to man. However, this is possible to predict by use of simpler *in vitro* methods (Tannergren et al., 2009). Regarding solubility limitations, data are complex, due to the influence of formulations and aspect of regional intestinal metabolism, which makes clear conclusions hard to be drawn. Regarding regional variation of intestinal metabolism, it seems that there is a clear species difference but this needs to be further elucidated. Overall, the dog has shown a reasonable good resemblance with humans regarding regional bioavailability data but if it offers any clear advantage with regard to predictive value on top of simpler *in vitro*/*in silico* approaches remains to be verified.

8.4.3. Rat model

Data on regional absorption comparison versus man is less well studied for the rat despite the fact that it is a simpler model. The work by Ungell et al. using rat tissue from different regions of the GIT showed consistently a reduction in permeability in colon for low permeability compounds whereas high permeability compounds, if anything, had a more similar permeation across the entire GIT (Ungell et al., 1998). Thus, the rat model can be expected to be a better discriminating system for drugs with passive permeability limitations. However, as metabolism differs between rat and man, both in regard of enzyme type and distribution, it is not likely that the rat model will correctly capture such effects in a manner relevant for man (Komura and Iwaki, 2011).

9. Excipients effects

9.1. Introduction

Excipients encompass a wide range of properties that are of importance for the resulting drug product ensuring stability, content uniformity and bioavailability of the incorporated APIs among many other factors. The total absorption of an API can be affected by the combination of excipients included in the formulation. This may be due to changes in the solid state of the API itself or the formulation characteristics of physical form (particle size, tablet hardness, porosity, hydrophilic properties of the total matrix, etc.) or the interactions between the components of the product (Panakanti and Narang, 2012).

Excipients may chemically react with the API, and thereby negatively influencing its availability for absorption. Such unintended incompatibilities have been reviewed lately by Bharate et al. (2010). For example, lactose led to degradation of acyclovir (Monajjemzadeh et al., 2009), amlodipine (Abdoh et al., 2004), metformin (Santos et al., 2008) and other amine-compounds in compatibility studies, whereas PVP led to degradation of oxprenolol (Botha and Lötter, 1990) and sulfathiazole (Voigt et al., 1984). Inclusion of magnesium stearate has shown to decrease the stability of moexipril hydrochloride and beta-lapachone, potentially by increasing the humidity in the formulation (Cunha-Filho et al., 2007; Stanisz et al., 2013). Conventional and modern QbD formulation development strategies will generally avoid such incompatibilities to occur in drug products.

From a biopharmaceutics point of view, functional excipients may exert a number of well-known effects including enhancement of wettability, dissolution rates and even solubility of the incorporated active ingredients. The solubility of an API is determined by its physical–chemical characteristics including its polymorphic form. This polymorphic form may be stabilized by addition of specific excipients (Singhal and Curatolo, 2004; Telang et al., 2009). Particle size is a determinant for the dissolution rate and the effective surface area for dissolution may also be influenced by excipients (Vialpando et al., 2011). Chemical or physical derivation of the API itself may be obtained during production of the active substance and/or of the finished product. See the section on models for API-formulation approaches later in this review for more examples in this context.

There are also less well-documented effects that may affect the bioperformance of a drug product such as modulation of intestinal transit times, interference with drug metabolizing enzymes and other constituents of the GI-tract, e.g. bile salts, which may in a unique way affect the absorption of a drug. Effects on physiological conditions and processes involved in drug absorption may lead to changes in the absorption profile.

Excipient effects on bioavailability are best shown by *in vivo* bioavailability studies in humans. Knowledge of these excipient effects is especially relevant in the context of comparative studies: formulation A versus formulation B containing different excipients. In many situations, comparative bioavailability studies are needed for new formulations to demonstrate equivalence. Such relative bioavailability/BE studies are not only relevant in the context of generic oral drug products, but also in the cases of a change in composition of an existing product. Understanding of intended or unintended effects of excipients on the pharmacokinetics of a drug substance is particularly interesting from the perspective of waiving *in vivo* BE studies for IR dosage forms.

Current EU Guidance on the investigation of BE states that BE studies for oral solutions of multisource drug products may be waived (EMA, 2010). However, if the excipients in the dosage forms involved affect GI transit (e.g. sorbitol, mannitol), absorption

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(e.g. surfactants or excipients that may affect transport proteins), *in vivo* solubility (e.g. co-solvents) or *in vivo* stability of the active substance, a BE study should be conducted, unless the differences in the amounts of these excipients can be adequately justified by reference to other data (EMA, 2010).

A BCS-based biowaiver may be considered for BCS class I and III (in the EU) drug compounds. As a general rule, for both BCS class I and III drug substances, well established excipients in usual amounts should be employed and possible interactions affecting drug bioavailability and/or solubility characteristics should be considered and discussed. A description of the function of the excipients is required with a justification whether the amount of each excipient is within the normal range.

The following overview will go through the physiological processes that a solid oral dosage form encounters after oral administration and give examples on how excipients may modulate these processes. It will discuss how the knowledge of these excipients is applied from a regulatory point of view and when mathematical models may be applied, especially in the context of biowaivers.

9.2. Modulation of drug release from dosage form: disintegration, dissolution and solubility

Drug release from the dosage form is essential for drug absorption as this will make the API available for absorption. The use of specific disintegrants to facilitate drug release is well-established for IR oral dosage forms. For example, the inclusion of sodium bicarbonate as disintegrant in the formulation led to a more rapid absorption of ibuprofen in humans compared to a formulations including aluminium hydroxide (Hannula et al., 1991). An enhancement in carbon dioxide production led to enhanced *in vivo* disintegration of the capsule, enhanced *in vivo* dissolution of the drug and enhanced gastric emptying rate. Examples of excipients that have been shown to improve *in vitro* dissolution rates are microcrystalline cellulose, D-glucosamine hydrochloride and PEG 6000 (Al-Hamidi et al., 2010; Alsaidan et al., 1998; Vijaya Kumar and Mishra, 2006). However, some super disintegrants have been described to interact with drugs, leading to decreased *in vitro* dissolution, although these interactions do not seem to affect the *in vivo* performance of the formulation (Fransén et al., 2008; Narang et al., 2012).

Excipients can also improve the solubility and dissolution of APIs via different mechanisms, e.g., solubilization, precipitation and supersaturation, as addressed elsewhere in this review. Cyclodextrins have for example been studied with the aim to increase the solubility and thereby the bioavailability of *in vivo* in different animal models by complexation of the drugs, e.g., griseofulvin, cinnarizin, glibenclamide, ibuprofen and nifedipine (Dhanaraju et al., 1998; Jarvinen et al., 1995) (Emara et al., 2002; Nambu et al., 1978; Savolainen et al., 1998). In regulatory practice however, cyclodextrins are not widely applied. The FDA Inactive Ingredients Database lists only one oral preparation in which hydroxypropyl-β-cyclodextrin is applied and seven parenteral products including cyclodextrines (FDA, 2013). Having said that, a number of cyclodextrin-containing oral dosage forms are available in worldwide formularies (Davis and Brewster, 2004).

On the other side, several studies have shown that excipients may also negatively affect the solubility of an API. In a recent study an interaction between SLS with intrinsic intestinal mixed micelles composed of bile salts and lecithin has been shown (Buch et al., 2010, 2009). The interaction led to a destruction of the micellar structure and a decrease in solubility of the low water soluble fenofibrate by SLS. Thus, instead of increasing the solubility of the drug by the addition of surfactant, the opposite effect was provoked with a concomitant decrease in the bioavailability of the SLS containing fenofibrate formulations (Buch et al., 2010, 2009).

Complex formation may reduce the availability of the API for absorption and consequently reduce its bioavailability. For instance, PEG 4000 formed insoluble complexes with phenobarbital, leading to decreased permeation across excised rat intestine (Singh et al., 1966). A reduction of bioavailability of the API was also observed when Tween 80 and sodium lauryl sulfate were combined with chlorpromazine and calcium salts and for magnesium carbonate with tetracycline (Chin and Lach, 1975; Nakano, 1971; Zak et al., 1978). This might have been caused by a complex formed between the API and the excipient(s). Complex formation was also postulated as mechanism for the interaction of calcium sulfate with phenytoin. When calcium sulfate was replaced by lactose the bioavailability of phenytoin increased so that the maximal safe plasma concentration was exceeded. However, additional studies did not confirm this mechanism and other authors suggested that the higher hydrophilicity of lactose as compared to that of calcium sulfate promoted increased dissolution of phenytoin. Nevertheless, other authors reported interactions of phenytoin with antacids and summaries of product characteristics of phenytoin include a warning that concomitant use of antacids or calcium salts may reduce the absorption of phenytoin (Bochner et al., 1972; Cacek, 1986; Chapron et al., 1979; Garnett et al., 1980).

The calcium sulfate-phenytoin data were correlated to result in bioinequivalence. However, in other cases there is often a lack of information on the magnitude of the excipient effect on the *in vivo* bioavailability of a specific API. The FDA Inactive Ingredient Database includes levels of excipients used in combination with the administration route (FDA, 2013). However, as the API included in the concerned dosage form is not listed together with these amounts, it is not possible to correlate the study data directly to levels actually used or to deduce safe levels from it. Additional information on the quantitative composition of bioequivalent products would be necessary to conclude on safe levels in combination with specific APIs.

9.3. Modulation of stomach physiology

The characteristics and volume of the pharmaceutical product as present in the stomach will determine the subsequent process steps. The authors are not aware of excipients that have been shown to change the function of the stomach physiology i.e. having an effect on the volume of the stomach itself or of the mechanistic aspects of stomach motility. Excipients affecting the luminal conditions including the stomach content are discussed below. The volume of the excipients, their liquid or solid state and perhaps also the effects on the viscosity of the stomach content may affect the gastric emptying time. See description of the stomach physiology in this review for information on relevant mechanisms in this context.

Strategies to increase residence time of an API in the stomach include the use of bioadhesive microspheres that slow intestinal transit and gastroretentive dosage systems (Ahmed and Ayres, 2007; Davidovich-Pinhas and Bianco-Peled, 2010; Davis, 2005; Prajapati et al., 2013). Small particles with bioadhesive properties and sometimes also floating capacity (on stomach content) are used to physiologically and physically reduce the gastric emptying. Positively charged molecules are thought to adhere to the negatively charged sialic acid groups on the gastric mucus. Strategies to enhance gastroretention may also make use of an enlarged object size and include swelling and floating agents. An example of such a formulation are superporous hydrogels formed *in situ* from polyethylene oxide with swelling capacity in combination with hydroxypropyl methylcellulose (HPMC) as sustained release matrix have been investigated (Kousar et al., 2013).

Formulations including gastroretentive mechanism are usually tested based on their specific physical characteristics using

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in vitro techniques which may include *in vitro* release testing as well as floating/buoyancy tests, the latter demonstrating the capacity of the dosage form to float on the gastric contents. However, an *in vivo* performance test in human or animal models is usually needed. Such investigations are generally carried out using radiology or scintigraphy visualization, endoscopic techniques or magnetic monitoring to show the location of the formulation or alternatively ^{13}C octanoic acid breath testing to determine the gastric residence time (Prajapati et al., 2013). See also the section above on imaging techniques.

9.4. Modulation of intestinal surface area

Morphological changes to the structure of the intestinal surface of the GI tract may change the absorption of drugs by changing the effective surface for absorption. Surfactants have been shown to cause (reversible) mucosal damage resulting in enhanced absorption of co-administered APIs. Rat intestinal perfusion studies also showed that co-administration of a mucolytic agent and a non-ionic surfactant improved the intestinal absorption of poorly absorbed hydrophilic (Swenson et al., 1994; Takatsuka et al., 2006, 2008) compounds. Histological evaluation also showed mucosal damage which is therefore thought to play a role in the observed absorption enhancement. See also below for more examples of permeability enhancers.

9.5. Modulation of pH of the GIT

Excipients may interact with the luminal components and thereby indirectly influence the dissolution and absorption of an API. For example, sodium bicarbonate increased the bioavailability of the acid-labile drug erythromycin acistrate in humans (Marvola et al., 1991), probably due to an increase in the gastric pH. The effects of excipients on the gastric pH could be simulated by *in vitro* tests, for example by simulation based on known compounds pH-stability profiles and the assumed residence times in the stomach. There also exist artificial stomach-duodenum models which incorporate the stomach to duodenum transit step with a concomitant shift in pH (Carino et al., 2010). However, so far animal and human studies provide the best measures of these potential effects. In the extrapolation from animal to humans it is however important to account for differences in animal GI physiology compared to man, as described in the sections above.

9.6. Modulation of GI transit and motility

The residence time and transit speed of an API are determined by the GI motility, which can be influenced by e.g., food intake or composition of the formulation.

Examples of excipients affecting gastric emptying time are described above. Another commonly discussed phenomenon is the reduction of the SITT and the consequences for absorption of drugs (Yuen, 2010). Well-known excipients that reduce the intestinal transit time in humans include the alcohol monosaccharides mannitol and xylitol as well as lactulose (Adkin et al., 1995a,b; Read et al., 1982; Salminen et al., 1989; Staniforth, 1989). Other examples are sodium acid pyrophosphate, polyethylene glycol and sorbitol (Adkin et al., 1995a; Basit et al., 2001, 2002; Chen et al., 2007; Chusid and Chusid, 1981; Islam and Sakaguchi, 2006; Koch et al., 1993; Payne et al., 1997; Schulze et al., 2003). The EMA pointed out that, since there is no information on the actual threshold of an effect of sorbitol on the PK of highly permeable drugs, strict compliance with the BE guideline is recommended, i.e., quantitative differences are not accepted in the context of biowaivers (EMA, 2010, 2013). Other excipients that are known to enhance GI motility are mentioned in Table 5. There are also excipients that increase the intestinal transit time such as oleic acid and lipids in general (Dobson et al., 1999; Martinez et al., 1995; Pilichiewicz et al., 2006). Formulations containing large amounts of lipids, like self-emulsifying systems, liposomes or micelles have the potential to modulate of intestinal transit times, especially for digestible lipids (Porter et al., 2004).

9.7. Modulation of GI fluids

Excipients may also affect the composition of the matrix in which the drug is transported through the GI tract. Lipid excipients in conjunction with the role of the animal model and its gastrointestinal fluid composition in characterizing the performance of lipid excipients containing dosage forms has been reviewed elsewhere (Hauss, 2007). Mucus formation may also be influenced by the presence of excipients; mucolytic agents are discussed above in the context of the intestinal surface area. Osmotic effects are discussed in the section on transit time, but could also be considered as an effect of modulation of the GI fluid content.

Table 5
Examples of modulators of residence time and/ or transit speed.

Excipient/modulator	Drug/marker compound	Model	Observed effect	Reference
Sodium alginate/karay gum gel	Barium sulfate	Rat/mice	Gastric retention	Foster et al. (2012)
Gellan and sodium alginate	Paracetamol	Rabbit and Rat	Gastric retention	Kubo et al. (2003)
Unfolding multilayer polymeric films	Riboflavin	Beagle dogs	Gastric retention	Klausner et al. (2002)
Swellable polymer films	Riboflavin	Dogs and Human	Gastric retention	Ahmed and Ayres (2007)
Polycarbophil with albumin	Chlorothazid	Human	Gastric retention	Longer et al. (1985)
Poly(acrylic acid), in gelatin microspheres	Oxprenolol hydrochloride	Rat	Gastric retention	Preda and Leucuta (2003)
Sodium bicarbonate	Aluminium hydroxide	Human	Reduced gastric emptying time	Hannula et al. (1991)
Sodium acid pyrophosphatee	Ranitidine	Human	Decreased small intestinal transit time	Adkin et al. (1995a), Koch et al. (1993)
Alcohol monosaccharides mannitol, xylitol and lactulose	–	Human	Decreased small intestinal transit time	Adkin et al. (1995a,b), Read et al. (1982), Salminen et al. (1989), Staniforth (1989)
PEG 400	Ranitidine	Human		Basit et al. (2001, 2002), Schulze et al. (2003)
Sorbitol	Ranitidine and metoprolol	Human		Chen et al. (2007), Chusid and Chusid (1981), Islam and Sakaguchi (2006), Payne et al. (1997)
Oleic acid	–	Human	Increased intestinal transit time	Dobson et al. (1999)
Lipids	–	Human	Enhanced gastrointestinal motility	Martinez et al. (1995), Pilichiewicz et al. (2006)

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9.8. Modulation of metabolism and intestinal degradation processes

Pharmaceutical excipients may interact with metabolic enzymes in the GI tract. Particularly nonionic surfactants and polymers have been shown to inhibit CYP activity. Examples of excipients with documented *in vitro* effect on CYP3A include different types of polyethylene glycol, Tween, Cremophor, Triton x, SLS, solutol, Lecithin and Vit C (Bittner et al., 2002; Bravo Gonzalez et al., 2002; Christiansen et al., 2011; Rao et al., 2010; Ren et al., 2009, 2008; Tompkins et al., 2010; Wandel et al., 2003). PEG 400 also inhibited CYP3A metabolism of Digoxin and Verapamil in excised rat jejunum (Johnson et al., 2002). Another type of excipient, β-cyclodextrin inhibited CYP3A4 and CYP2C19 *in vitro* on cDNA expressed human cytochrome P-450 (Ishikawa et al., 2005). As shown by the above examples, the excipients vary in their inducing or inhibiting effects on CYPs. It is also conceivable that excipients affect other metabolising enzymes than CYPs, e.g., UGTs and SULT. *In vitro* digestion models are available that simulate the digestion of formulations in (parts of) the GI tract (Dahan and Hoffman, 2008; Mohsin, 2012; Versantvoort et al., 2004), but publications in the context of excipient effects are limited. *In vitro* tests to study specific enzyme interactions also exist, but are mainly used in the context of drug–drug interactions (EMA, 2012). Generally there is a lack of *in vivo* data on excipients inhibition effects, in particularly in humans, so extrapolations of *in vitro* effects to the *in vivo* situation are difficult.

A combination of carboxymethyl-starch excipients and enzyme inhibitors may enhance the gastroresistance and stability against gastrointestinal enzymes of an active substance in the GIT (De Koninck et al., 2010; Nassar et al., 2008). In addition to their potential role in increasing the solubility of the API as discussed above, cyclodextrines may form a non-degradable complex with the API thereby protecting it from degradation and increasing its bioavailability (Challa et al., 2005).

9.9. Modulation of membrane transport

Polarity of the molecule and characteristics like particle size determine the intrinsic permeability of the API. These aspects could also be modulated in presence of specific excipients. Micronisation to microparticles or even nanoscale particle size increases the contact surface of the active substance with the intestinal membrane. Ross and Toth reviewed the use of microparticles and nanoparticles containing heparins that enlarged the contact surface of the heparin (Ross and Toth, 2005). Biodegradable poly-caprolactone and poly(lactic-*co*-glycolic acid) and nonbiodegradable positively charged polymers such as Eudragit RS and RL were reported to enhance the absorption of heparin. The polymers were used alone or in 1:1 combination and both micro- and nanoparticles were shown to increase the bioavailability of heparin. The mechanism of the nanoparticles absorption enhancement was however not determined. Ross and Toth also described how polyanionic low molecular weight heparins (LMWH) were paired with polycationic lipophilic-core dendrons (PLCDs) (Ross and Toth, 2005). Lipoamino acids were designed to increase the lipophilicity of the complex. These PLCDs may also act by perturbing the cell membrane. Several successful studies in which the concept of lipophilic ion-pairing with diamines, triamines and lipoamino acids was shown were summarized (Ross and Toth, 2005). However, according to the authors, the variety of models (*in vivo* models like rat oral gavage, rat intraduodenal, mouse oral gavage, pig intraduodenal, rabbit oral gavage, monkey oral gavage, dog oral gavage and also Caco-2 cell monolayers and Ussing chamber) made it difficult to compare the results of different studies.

9.9.1. Paracellular route: tight junction modulation

Several authors reviewed possibilities for absorption enhancement by the paracellular pathway (Cano-Cebrian et al., 2005) and the implication of tight junction modulation for drug delivery (Salama et al., 2006). Numerous potential modulators have been investigated with the purpose of increasing permeability by opening tight junctions. For many of these compounds, the dose needed to reach an effect was so high that cytotoxicity limited the application and further explorations were stopped. This applied for example for calcium chelator EDTA, sodium dodecyl sulfate, cytochalasins, saponines and acylcarnitines. These compounds were tested mainly in cell culture models like Caco-2 and/or on intestinal mucosa (Maher et al., 2008), but some also in animal studies (see above at modulation of the intestinal surface).

Some permeability enhancers, such as medium-chain amphipathic fatty acid like sodium caprate and polymeric enhancers like chitosan and its derivatives, carbomers and thiolated polymers, reached the phase of *in vivo* studies in pre-clinical species, e.g., rats, pigs and dogs, as well as in the clinic (Cano-Cebrian et al., 2005; Maher et al., 2008; Thanou et al., 2001b).

Permeability can also be enhanced by making use of the structural similarity of the active substance and the enhancers. The absorption process of peptide drugs may be improved by peptide-based permeability enhancers, which being proteins themselves, share physicochemical properties, diffusion characteristics and stability issues with the therapeutic proteins being delivered (Maher et al., 2008). However, oral formulations are not available as far as known to the authors of this paper. Clinical studies suggest that there is no toxicity of concern and the absorption-promoting effects were transient and complete in <1 h. Other examples of compounds which have been studied for their effects on the paracellular transport are listed in Table 6.

9.9.2. Transporter-mediated absorption

The influence of excipients on transporter-mediated absorption (Table 7) has lately been reviewed by Grube and Langguth and by Goole et al. (Goole et al., 2010; Grube and Langguth, 2007). Maher described cell-penetrating peptides (CPP's) that have been studied to improve the delivery of protein drugs in the target cells (Maher et al., 2008). These cationic amphipathic peptides include poly-L-lysines, poly-L-arginine. These two peptides were mainly studied for delivery in the nasal or tracheal epithelial cells. Transportan and penetratin are two other CPP's having cell-penetrating effects of which analogs are thought to be potentially interesting permeability enhancers.

9.9.3. Passive diffusion and endocytosis

Passive diffusion could be affected by changes in either the apical or the basolateral membrane. Guan et al. describe an investigation of the mechanisms of improved oral bioavailability of bergenin by complexation of bergenin with phospholipid and conclude that the complex could transport across enterocytes by both passive diffusion and active transport by receptor-mediated endocytosis (Guan et al., 2013). The authors used experimental models such as the *ex vivo* everted rat gut sac model and *in vitro* Caco-2 cell monolayers and the effect was limited (Guan et al., 2013).

9.10. Models for testing excipient effects and their application for biowaivers

To study the effects of excipients on the bioavailability the pharmaceutical industry and academia apply different techniques, each with their own limitations.

The current regulatory biowaiver guidance limits itself to the first step in the GI absorption of the API from an immediate release solid oral dosage form: drug release (EMA, 2010). As the review above

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Table 6
Examples of paracellular transport modulators and marker compounds.

Excipient/modulator	Drug/marker compound	Model	Observed effect	Reference
EDTA	PEG4000	Caco-2	Enhanced permeability	Tomita et al. (1994)
EDTA	Iron	Caco-2	Increased absorptoin	Kibangou et al. (2008)
Sodium caprate	Macromolecules	Intestinal cells	Increased permeation	Krug et al. (2013)
Sodium decanoate	Antisense oligonucleotides	Pigs	Enhanced bioavailability	Raoof et al. (2002)
Sodium decanoate	Cefmetazole	*In situ* loop study	Increased jejunal absorption	Tomita et al. (1992)
Sodium decanoate	Ampicillin	Human	Absorption after rectal administration increased	Lindmark et al. (1997)
Sodium decanoate in GIPET	Acycline	Human	Oral	Amory et al. (2009)
Palmitoyl carnitine	Mannitol and PEG 4000	Caco-2 and IEC-18	Enhanced permeability	Duizer et al. (1998)
Palmitoyl carnitine	Cefoxitin	Dogs and rats	Enahanced bioavailability	Sutton et al. (1993)
Chitosan-coated nanoparticles	Insulin	Rats		Lin et al. (2007)
Chitosan	Oxaprozin	Caco-2	Enhanced permeability	Maestrelli et al. (2011)
Chitosan hydrochloride and glutamate salts	Peptides	*In vitro*	Enhanced absorption	Kotzé et al. (1997)
Chitosan hydrochloride		Rats	Enhanced absorption	Luessen et al. (1996)
Trimethyl chitosan	Octreotid acetate	Pigs	Intraduodenal application increased bioavailablity	Thanou et al. (2001c)
N-sulfonato-N,O-carboxymethylchitosan	Macromolecules	Caco-2	Enhanced permeation	Thanou et al. (2007)
Mono-N-carboxymethyl chitosan	Low molecular weight heparin	*In vitro* and *in vivo* rat model	Increased intestinal absorption	Thanou et al., 2001a
N-trimethyl chitosan (TMC)	Insulin loaded nanoparticles	Caco-2 and excised rat jejunum	Increased permeation	Sandri et al. (2010)
N-trimethyl chitosan (TMC)	Buserelin and octreotid acetate	Rat and pig	Enhanced bioavailability	Thanou et al. (2007, 2001c)
Sodium lauryl sufate	Cefradoxil	Rat duodenum	Enhanced absorption	Sancho-Chust et al. (1995)
Tetradecylmaltoside	Enoxaparin	C2BBe1-cells and Rats	Enhanced absorption	Yang et al. (2005)
Octylglucoside	Insulin	Caco-2 and T84 monolayers	Enhanced permeation	Tirumalasetty and Eley (2006)
Chenodeoxycholates	Oligonucleotides	Rat jejunum and ilem	Enhanced paracellular diffusion	Tsutsumi et al. (2008)
Sodium taurocholate	Insulin	Caco-2	Increased permeation	Degim et al. (2004)
Thiolated polycarbophil/glutathione	FITC dextran and sodium fluorescein	Excised rat jejunum		Perera et al. (2011)
Other thiolated polymers (e.g. chitosan-cysteine and chitosan-4-thio-butylamidine)	Hydrophilic compounds	*In vitro*	Enhanced permeability	Bernkop-Schnurch et al. (2004), Bernkop-Schnürch et al. (1999)
Butylated methacrylate copolymer (Eudragit E®)	Mannitol, Talinolol, and Trospium	Caco-2	Enhanced permeability	Grube et al. (2008)

shows, animal models are commonly applied by pharmaceutical industry to test differences in drug release from pharmaceutical formulations. However, in the context of BE questions, these models do play a limited role: animal models are not approved as models for biowaivers. From a regulatory perspective, a human volunteer is the only acceptable 'model' for comparative bioavailability testing when biowaiver conditions are not met and in absence of an *in vivo in vitro* correlation of the dissolution versus plasma data.

Cell culture models for testing permeability effects are well-known and *in vitro* digestion models and enzyme interaction models also exist. However, these are not approved to confirm BE of formulations either. The GI transit of APIs can be studied using different *in vivo* techniques, but comparative *in vitro* models validated for their biorelevance of this parameter are not known to the authors. In conclusion, apart from the dissolution test, there is no comparative *in vitro* test model validated and approved for comparative testing of disposition effects.

The regulatory guidance is limited as to allowed (difference in) levels of excipients: as a general rule for BCS class I and III drug substances well-established excipients in usual amounts should be employed and possible interactions affecting drug bioavailability and/or solubility characteristics should be considered and discussed. Even in the case of Class I drugs it is advisable to use similar amounts of the same excipients in the composition of test like in the reference product. If a biowaiver is applied for a BCS class III drug substance, excipients have to be qualitatively the same and quantitatively very similar (EMA, 2010). Effects on disintegration or dissolution could be tested by the pharmacopoeial models; however, no specific excipient information or other details are given on the evaluation of any test outcome.

9.11. Gap analysis and consequences for biowaivers

Excipients may theoretically affect the GI absorption of API in many ways. However, the *in vivo* relevance of data obtained by models is not always clear.

In addition, the current usability of the available models to detect excipient effects in the context of regulatory applications in the EU is limited. Only one *in vitro* model is sufficiently validated and approved from a regulatory point of view: the dissolution test. However, many other models exist and many relevant data on excipient effects are available at pharmaceutical industry. Little of this knowledge obtained in drug development studies is currently translated into regulatory guidance on excipients. Authorities may, therefore, seem unnecessary restrictive in the acceptance of differences in excipients. However, it should also be noted that authorities do not dispose of these, mostly confidential, company data and development of regulatory acceptable models depends on the availability of public data and shared knowledge.

The available guidance on biowaivers shows that EU regulators are in principle open to submissions including adequate justification of full BCS based biowaivers or waivers in the context of a change in composition of the product. For BCS class I biowaivers

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Table 7
Examples of modulators of transporter mediated absorption.

Excipient/modulator	Drug/marker compound	Model	Observed effect	Reference
Acconon E	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
Cholesterol	cytotoxic agents	?	ATPase activity of P-gp	Shu and Liu (2007)
Cremophor EL	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
Cremophor EL	Rhodamine 123	Caco-2 and rat intestinal membranes	Increased transport	Rege et al. (2002)
Cremophor EL	Taxol	Caco2	Increased transport	Hugger et al. (2002b)
Cremophor RH 40	Digoxin	Human	Reduced bioavailability	Tayrouz et al. (2003)
Imwitor 742	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
Labrasol	Celiprolol	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
Miglyol	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
MS-310, MO-310 and MS-500	Ceftibuten	BBM Vesicles	Increased uptake	Koga et al. (2000)
N-dodecyl-β-D-maltopyranoside	Rhodamine 123	Rat intestinal membrane	Increased transport	Shono et al. (2004)
PEG 300	Taxol	Caco-2 and MDR1-MDCK cells	Increased transport	Hugger et al. (2002a)
PEG 400	Digoxin	Rat jejunal tissue	Inhibition of efflux	Johnson et al. (2002)
Pluronic L61	Vinblastine	LLC-MDR1 cells	Increased transport	Evers et al. (2000)
Different types of Pluronics	Rhodamine 123	KBv cells	Increased uptake	Batrakova et al. (1999a)
Pluronic P85	Different substances	Caco-2- and BBMEC-cells	Increased transport	Batrakova et al. (1999b)
Polysorbate 20	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
Polysorbate 20	Ceftibuten	BBMV vesicles	Increased uptake	Cornaire et al. (2004)
Polysorbate 40	Ceftibuten	BBMV vesicles	Increased uptake	Koga et al. (2000)
Polysorbate 80	Rhodamine 123 and other substances	Caco2-cell-monolayers and rat intestinal membrane	Increased transport	Rege et al. (2001)
Sodium lauryl sulfate	Different substances	Caco-2	Increased transport	Cornaire et al. (2004)
Softigen 767 and SS-500	Digoxin	Everted rat gut sac	Increased uptake	Cornaire et al. (2004)
TGPS 1000	Talinolol	Caco-2 and human	Increased trasnport and increased AUC	Rege et al. (2002)
TGPS	Colchicine and doxorubicin	G185 cells	Decreased permeation	Dintaman and Silverman (1999)
TGPS 800 and TS-500	Ceftibuten	BBMV vesicles	Increased uptake	Koga et al. (2000)

'qualitative differences' are acceptable, if appropriately justified and provided that excipients that might affect the bioavailability are qualitatively and quantitatively the same. In case of a change in composition, 'minor changes' in excipient content may be accepted based on dissolution data only i.e. without addressing other steps in the absorption process. In such a case, the classification of the change as 'minor' is to be assessed on a case-by-case basis.

To avoid the need for comparative testing of each difference in composition and clarify the classification of changes as 'minor' or 'major', publication of information of the effects of specific excipients in combination with specific APIs seems useful. Formulation development handbooks advise on usual concentrations of well-known excipients but do not indicate 'no effect levels' of excipients or 'safe windows' in the context of their effect on the bioavailability of active substances. And, information on the quantitative composition of pharmaceutical products is confidential while actual levels of excipients are not clear in the EU. So far, the FDA Inactive Ingredients Database is used by some authors to refer to actually approved levels of excipients, e.g. in the biowaiver monographs published by the FIP on http://www.fip.org/bcs. A public database on excipient effects could lead to cut-off values for specific excipient effects and may allow building up 'safe excipient ranges' or a 'safe space' per excipient. In addition, such database could clarify which excipients are known to physiologically affect the bioavailability of APIs and identify 'suspect excipients' or 'bioavailability modulators' in the context of biowaivers.

10. Food effects

10.1. Introduction

Altered human drug PK in the presence of food (including dietary supplements and nutraceuticals) has been extensively examined from a scientific and regulatory perspective (Abdel-Rahman et al., 2011; Boullata and Hudson, 2012; Chan, 2002; Custodio et al., 2008; Fleisher et al., 1999; Genser, 2008; Joshi and Medhi, 2008; Mills et al., 2005; Rodriguez-Fragoso et al., 2011; Schmidt and Dalhoff, 2002; Singh, 1999; Welling, 1977; Won et al., 2010, 2012).

Food effects are derived from two basic principles of mechanisms: the impact of the meal's content itself and the postprandial changes of GI physiology. From a physiological perspective, food intake, compared to the fasted state, can particularly provoke (Fleisher et al., 1999; Welling, 1977):

- Changes of visceral blood and lymph flow.
- Intraluminal composition in qualitative and quantitative terms.
- Time- and region-specific alterations of hydrodynamics and mechanical forces within the GI tract.
- Modulation of drug metabolizing enzymes and transporters resulting in distinct absorption, distribution and elimination characteristics.

Despite these physiological and physicochemical variations, based on the BCS, several reviews stated the absence of food effects on the oral drug bioavailability for BCS class I compounds (Custodio et al., 2008; Fleisher et al., 1999). Likewise, the positive effect of high-fat meals on the absorption of non-ionizable and weak acidic BCS class II drugs in immediate-release formulations seems to be predictable (Custodio et al., 2008; Fleisher et al., 1999). Including transporter-related aspects according to the BDDCS, Custodio et al. (2008) hypothesized distinct trends to be expected for the oral bioavailability of BDDCS class I and II compounds with concomitant food intake (Custodio et al., 2008). For modified-release formulations, BCS class III and IV drugs, however, the interplay of various parameters in the fed state can yield more complex scenarios – *in vivo* models could be of great value in these cases, presuming that the species characteristics regarding certain physiological parameters are kept in mind.

Categorizing different effects of food based on the biopharmaceutical processes that are affected, the next paragraphs of this review focus on the use of common laboratory animals to evaluate food effects *in vivo*.

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10.2. In vivo assessment of food effects on the pharmacokinetics of orally administered drugs

10.2.1. Disintegration, dissolution, diffusivity and intra-luminal reactions

Modified dissolution and precipitation characteristics via solubilization (Charman et al., 1993; Rolan et al., 1994), pH alteration (Carver et al., 1999; Zimmermann et al., 1994) and reactions with meal components (Huupponen et al., 1984; Jung et al., 1997; Neuvonen et al., 1991) have been proposed to explain changes of human drug pharmacokinetics in the presence of food. To elucidate these mechanisms, intestinal perfusion techniques can be of great value. Fuse et al. (1989), for example, investigated the influence of pectin as dietary fiber by means of intestinal perfusion in rats and humans, concluding that not binding of bile acids, but expansion of the unstirred water layer is the major factor leading to decreased absorption of linoleic acid and glucose (Fuse et al., 1989). With a porcine intestinal perfusion model, Persson et al. (2008) showed that solubilization rather than P-gp inhibition is responsible for the positive food effect observed for danazol as a model compound for low solubility drugs (Persson et al., 2008).

Among whole animal models, the dog model seems most appropriate to investigate food effects on the disintegration and dissolution of oral solid formulations (Abrahamsson et al., 2004; Wu et al., 2004), due to physiological and dosage form related advantages as well as the suitability to receive infrequent large meals on command (Lentz, 2008; Sutton, 2004). In contrast, overprediction of the solubility enhancing impact of food (Campbell and Rosin, 1998; Humberstone et al., 1996; Paulson et al., 2001; Xu et al., 2012) and insufficient differentiation between fasted and fed state with respect to dose dumping behavior of a matrix tablet formulation have been reported (McInnes et al., 2008). Consequently, higher bile salts level (Carlsson et al., 2002; Dressman, 1986; Kalantzi et al., 2006), lower gastric acidity in fasted state opposing lower postprandial pH (Dressman, 1986; Lui et al., 1986; Mahar et al., 2012; Sagawa et al., 2009) (Table 1) and higher mechanical forces in the in the stomach (Kamba et al., 2001, 2002) are some physiological factors that need to be considered when extrapolating from canine disintegration and dissolution data to human. Looking at the controversial comparative solubility data in fed intestinal aspirates (Kalantzi et al., 2006; Persson et al., 2005) and the variety of applied test meals reviewed by Lentz et al. (Lentz, 2008), meal volume and composition should be standardized to improve correlations. Simulating human stomach pH by stimulating gastric acid secretion, e.g. with pentagastrin, is an additional approach that requires further validation for fasted versus fed state comparisons (Ajayi et al., 1999; Akimoto et al., 2000; Fancher et al., 2011; Lentz et al., 2007; Polentarutti et al., 2010).

Other species, e.g. rats (Morita et al., 2006), rabbits (Dongowski et al., 2005) and pigs (Grove et al., 2007), are less represented throughout literature concerning the impact of food on GI absorption. The pig might be an alternative to the canine model for solubility related food effects (Grove et al., 2007) as the biliary system and pancreatic duct of minipigs, for example, are more consistent with human data in size and function (Kararli, 1995; Swindle and Smith, 1998). In principle, the monkey could also serve as an animal model to study the effect of food on drug absorption, albeit reported human-primate discrepancies with respect to intestinal metabolism which is addressed in another paragraph of this review (Ikegami et al., 2003). Still, apart from ethical aspects as well as difficulties in animal supply, handling and high costs, defining a suitable standard meal yielding postprandial pH profiles comparable to human appears to be one of the major obstacles for the use of the monkey model for predicting food effects on absorption. In spite of quantitative discrepancies probably attributable to the different measuring techniques, two studies indicated that the overall time needed to return to basal acidity after food digestion is significantly longer in monkey than in human (Chen et al., 2008; Kondo et al., 2003a). This was so even after replacing the standard biscuit-type meal by fruits or jelly type food, respectively (Kondo et al., 2003b).

Summarizing the available reports, the effect of food on drug solubility and stability has been vastly investigated, mostly using dogs, GI aspirates and perfusion studies, whereas the dietary impact on formulation integrity and diffusivity of the released drug to the GI mucosa is scarcely described for any species. There is evidence that the poor correlation of food-induced enhancement of solubility with dissolution rates *in vivo* might be ascribed to reduced diffusivity of the forming mixed micelles (Charman et al., 1997; Lennernäs, 2007c). Moreover, studies in dogs and humans implicated that postprandial increased viscosity in the upper GI tract modulated absorption profiles by decreasing gastric emptying times, dissolution rates and diffusivity of drugs with pronounced region-specific absorption (Pao et al., 1998; Reppas et al., 1998). Hence, combining methods to assess mechanical forces (Marciani et al., 2001b; McInnes et al., 2008), media conditions e.g. viscosity (Marciani et al., 2000, 2001b, 1998) and fluid volumes (Schiller et al., 2005) as well as *in vivo* dosage form performance (Sutton, 2004) should be considered in future *in vivo* food effect studies.

10.2.2. Permeability and region-specific absorption

With respect to para- and transcellular transport, food may modulate intestinal permeability by direct, bile salts-induced or microflora-mediated changes in membrane integrity and fluidity (Bagchi et al., 1998; Kvietys et al., 1991; Ten Bruggencate et al., 2006), altering the pH dependent extent of ionization (Charman et al., 1997; Marasanapalle et al., 2009), forming mixed bile salt micelles thus reducing the free fraction of lipophilic drugs (Charman et al., 1997; Poelma et al., 1991) and increasing fluid flux facilitated passive diffusion (Kitazawa et al., 1978; Lane et al., 2006; Lu et al., 1992; See and Bass, 1993). The latter has been subject of controversial discussion since contrary to data generated in rodent models, the nutrient-induced solvent drag effect on intestinal permeability has not always been observed in human (Fagerholm et al., 1995; Fine et al., 1993; Lennernäs, 1995; Nilsson et al., 1994). This apparently is due to the complexity of factors involved such as molecular weight, ionization, segmental difference, ion partitioning effects, bulk flow transport towards the intestinal wall, tight junction contribution, motility and relative magnitude of transcellular versus paracellular water movement (Fagerholm et al., 1999; Johno and Kitazawa, 1985; Lennernäs, 1995, 1998; Pappenheimer and Reiss, 1987; Soergel, 1993).

There are evident physiological and methodological discrepancies for estimating intestinal permeability between rats and humans, including about 4-fold higher thickness of the unstirred water layer in the rat (DeSesso and Jacobson, 2001; Hurst et al., 2007; Kararli, 1995), dynamic changes of the functional absorptive area as a function of the species, interspecies differences in villus tip osmolality and unknown transporter contribution, anesthesia effects and segmental distensions *in situ* (Bijlsma et al., 1995; Lennernäs, 2007a). Nevertheless reviews confirmed reasonable correlation between the jejunal permeability of rat and human for passively transported drugs, regardless of the permeability classification of the compounds (Lennernäs, 1998, 2007c), and the suitability of the rat model as a predictor of the fraction of drug absorbed in human (Chiou and Barve, 1998). For this reason, the rat is the most used model for determining permeability-related nutrient–drug interactions, either by using *in vivo* perfusion techniques or noninvasive differential urinary excretion approach (Lane et al., 2006; Lu et al., 1992; Schepens et al., 2008; See and Bass, 1993; Song et al., 2011; Suzuki and Hara, 2010).

Compared to the rat, the canine small intestine exposes a higher permeability to hydrophilic substances which is presumably

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related to differences in the paracellular pathway and villi morphology (He et al., 1998; Lennernäs, 2007a; Martinez et al., 2002; Sutton, 2004) resulting in a poor correlation of fraction of dose absorbed in man (Chiou et al., 2000). Similarly, studies conducted at whole-animal level indicated that paracellular absorption is higher in pigs than in rats, while the underlying mechanism is still unknown (Delahunty and Hollander, 1987; Lavin et al., 2007). Therefore, the use of pigs and dogs for nutrient-induced modulation of drug permeability is limited. Still, dietary effects need to be accounted for during breeding as certain food constituents may alter mucosal integrity and function in these mammals (Watson et al., 2006; Zhang and Guo, 2009).

Although the upper GI segments are considered to be the main site of absorption, distal regions including ileum and colon can significantly contribute to the overall absorption as well. Segmental single-pass perfusion (Fagerholm et al., 1997) and site-specific administration (Lindahl et al., 2004) have been applied to the rat intestine to investigate regional differences in absorption. Sakuma et al. (2007) demonstrated by using a rat model *in vivo* combined with surgical assessment of GI transit of food components that varying the administration site could help decreasing the negative food effect for several model drugs (Sakuma et al., 2007). Yet, unlike human, the absorptive surface area is more evenly distributed in the rat (DeSesso and Jacobson, 2002) and some inconclusive results e.g. regarding colonic permeability (Fagerholm et al., 1997; Hollander et al., 1989; Krugliak et al., 1994) along with solid dosage form related restrictions as mentioned elsewhere limit the use of the rodent model for the prediction of more complex absorption profiles. Moreover, despite the development of sophisticated intubation techniques to study regional absorption in human such as triple-lumen tubing and rectal perfusion (Gramatte, 1994; Lennernäs et al., 1995), directly *in vivo* obtained permeability values for human ileum and colon are lacking, most likely due to experimental difficulties (Lennernäs, 2007a). Hence, extrapolation from preclinical data to human is a delicate challenge for low permeability drugs and formulations for which the distal region is the preferable site of drug release and absorption. However, efforts are being made to develop more precise preclinical models for this purpose, e.g. by using GI transit time controlled beagle dogs for the successful estimation of the bioavailability of paracetamol from a sustained-release formulation (Yamada et al., 1995) or by introducing a canine colonoscopy model as a surrogate for human intubation studies exploring controlled-release formulation behavior (Sutton et al., 2006). All the same, the suitability of the dog for investigating modified-release formulation and site-specific absorption – with or without food – has been controversially evaluated (Akimoto et al., 1995; Cook et al., 1990; Ishibashi et al., 1999a,b; Kulkarni et al., 2012; Li et al., 2001; Pao et al., 1998; Sutton, 2004, 2009, 2006; Wu et al., 2004; Yamada et al., 1995). For extended release formulations, the porcine model might be preferred which can partly be explained by more similar GI surface area and transit times to human (Kulkarni et al., 2012; Lennernäs, 2007a). Despite some shortcomings in terms of quantitative estimation, the dog remains a useful model to explore underlying mechanisms of food effects on oral dosage form behavior, irrespective of dissolution (Wu et al., 2004) or permeation dependent regional differences in absorption (Li et al., 2001; Pao et al., 1998; Sutton, 2004). Nonetheless, especially for modified-release formulations and compounds with site-specific absorption, food-induced changes in GI transit times have to be taken into account which is addressed in the next paragraph.

10.2.3. Transit times

Food intake is known to influence GI transit times (GITT), e.g. by delaying gastric emptying time in dependence of volume, caloric content, stomach pH, viscosity, lipid digestion and timing relatively to the interdigestive migrating motility complex (Collins et al., 1996; Davis et al., 1986; Fleisher et al., 1999; Kaniwa et al., 1988b; Lin et al., 1993; Meyer et al., 1985; Welling, 1977). Thus, postprandial conditions are often associated with altered absorption profiles of substrates and drug formulations with pronounced absorption window due to site-specific dissolution, instability, targeting concept et cetera (Gouda et al., 1987; Ishibashi et al., 1999a; Marathe et al., 1998; Pao et al., 1998; Sunesen et al., 2005; Yuen, 2010). For BCS Class I and III compounds which are rapidly absorbed in proximal parts, for example, gastric emptying is critical for the absorption rate, but not necessarily for the overall extent of absorption (Fleisher et al., 1999; Pao et al., 1998).

Although the SI transit times (SITT) is hardly affected by concomitant food consumption (Billa et al., 2000; Davis et al., 1986; Fleisher et al., 1999; Kararli, 1995; Kenyon et al., 1995; Yuen et al., 1993), some nutrient-induced feedback mechanisms on gastric emptying (Lin et al., 1993, 1992), species difference in the fasted state (Kararli, 1995) and the gastro-ileocaecal reflex (Fadda et al., 2009; Kerlin et al., 1982; Schiller et al., 2005) can have consequences on fasted versus fed state comparisons. The *in vivo* relevance of these alterations has to be evaluated in dependence of the dosage form – monolithic formulations, for example, have been reported to be more affected than multiparticulate preparations (Fadda et al., 2009; Mundy et al., 1989; Yuen et al., 1993).

Overall, substantial interspecies differences in GITT in fasted and fed state mitigate the accuracy in predicting food effects, as summarized in several reviews (Martinez et al., 2002; Martinez and Papich, 2009; Sutton, 2009). Moreover, uncertainties in capturing the known influence of meal viscosity, lipid digestion products and breed size, etc. on GITT (Bourreau et al., 2004; Ehrlein and Prove, 1982; Fix et al., 1993; Meyer et al., 1994) can impair the prediction quality of *in vivo* models.

Nutrient-induced alterations of GITT seem to be similar in qualitative terms, but highly variable in quantitative evaluations. Again, the dog is the most studied preclinical model in this aspect, showing rather poor correlation up to over-prediction of food effects due to more pronounced postprandial delay of gastric emptying and faster GITT in the fasted state as compared to human (Campbell and Rosin, 1998; Kaniwa et al., 1988a; Paulson et al., 2001) (Table 1). For the assessment of modified-release formulation behavior in both fasted and fed state, human-canine dissimilarities in bioavailability have also been partly attributed to distinct residence times in the targeted region, especially for monolithic dosage forms (Fix et al., 1993; Ishibashi et al., 1999a; Kabanda et al., 1994). The porcine model, too, has to be evaluated with caution, since its stomach residence time is remarkably longer than in human (Aoyagi et al., 1992), although the overall GITT time seems to be more comparable to human than that obtained from the canine model (Kararli, 1995; Kulkarni et al., 2012). Considering the rat which may be used for oral disperse formulations, there are indeed some similarities e.g. regarding the intestinal transit time (Hurst et al., 2007), but obviously, rodent models are inappropriate for large infrequent meals which is needed to simulate human dietary behavior. With respect to GITT in fasted and postprandial conditions, the monkey appears most suitable albeit some dissimilarities in SITT, according to Ikegami et al. (2003). Its application remains limited for reasons mentioned above, though.

Regardless of which *in vivo* model is employed, if food-induced alteration of the GI residence times of a dosage form occurs, the risk of subsequent changed stability, unexpected absorption pathways as well as modified metabolism and transport pattern should be taken into consideration which, in turn, varies across species.

10.2.4. Lymphatic uptake

Intestinal lymphatic transport is considered a relevant pathway to circumvent hepatic first pass extraction and for the delivery of

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certain antiviral, immuno-modulatory and anticancer drugs (Porter and Charman, 2001; Trevaskis et al., 2008; Yáñez et al., 2011). The contribution of lymphatic uptake to oral bioavailability can be estimated from physicochemical characteristics of the molecules, still, it is important to take account of the very low rate of lymphatic fluid transport – approx. 0.2% (v/v) – relative to portal blood (Charman et al., 1997). In general, fat-soluble vitamins, dietary or synthetic lipids and lipophilic peptide-like compounds are prone to absorption into the lymphatic system (Charman et al., 1997; Porter and Charman, 2001; Trevaskis et al., 2008; Yáñez et al., 2011).

The postprandial increase of luminal lipid concentration evidently favoured lymphatic uptake of the lipophilic prodrug testosterone undecanoat relative to testosterone resulting in higher systemic exposure of the former in the fed state (Bagchus et al., 2003; Frey et al., 1979). The absorption of halofantrine, a compound with remarkable positive food effect (Milton et al., 1989), has also been proven to be mediated by lymphatic transport (Porter et al., 1996). All the same, the interplay of bile salts and pH on solubility and extent of ionization should be considered when evaluating the potential of a drug to be associated to lipid digestion products for the subsequent transport into the lymph (Charman et al., 1997; Yáñez et al., 2011). The role of lymphatic transport is hardly sufficiently assessed by plasma concentration–time profiles only, since the increased lymphatic uptake after a high-fat meal may result in lower plasma levels of the drug in question (Charman et al., 1997; Porter and Charman, 2001). Therefore, animal models allowing sampling or exhaustive collection of lymph are indispensable to assess the contribution of lymphatic transport to enhanced postprandial bioavailability. Theoretical and practical aspects of the sophisticated techniques including advantages and limitations of the respective models have been described profoundly in reviews on this topic (Edwards et al., 2001; Trevaskis et al., 2008; Yáñez et al., 2011). Briefly, the conscious and unconscious, restrained or unrestrained rat models have been widely used to gain mechanistic understanding of lymphatic contribution to drug absorption (Charman et al., 1986; Jandacek et al., 2009; Porter et al., 1996; Turner and Barrowman, 1977). Larger animal models like dogs, pigs, sheep and rabbits as well as an indirect pharmacological approach avoiding lymph-duct canulation have also been developed (Dahan and Hoffman, 2005; Khoo et al., 1998, 2002, 2003; Shackleford et al., 2003; White et al., 1991; Yáñez et al., 2011). In summary, reports on lymphatic transport and veterinary drug absorption stated the more representative fasted and fed states of the porcine and canine model, but also outlined the general restriction in comparability of the models among each other and relative to human. This is mainly because of experimentally caused bias related to the variable surgical and anaesthetic methodologies and the overall insufficient knowledge of species difference in drug lymphatic uptake (Cook et al., 1998; Hurst et al., 2007; Martinez et al., 2002; Trevaskis et al., 2008; Yáñez et al., 2011). Hence, investigations on the impact of food with respect to lymphatic transport remain rather case-specific, and the relevance with respect to the drug systemic exposure *in vivo* can be considerably affected by interspecies differences regarding lymphatic flow and mechanism of lymphatic absorption.

10.2.5. Metabolism

Food may interfere with drug metabolism or enterohepatic recirculation, e.g. by inhibiting hydrolytic enzymes produced by intestinal bacteria (Schmidt and Dalhoff, 2002).

Metabolism-related food–drug interactions are highly dependent on the composition of the food, namely, they are mostly associated with fruits, vegetables, alcoholic beverages, teas and herbs (Rodriguez-Fragoso et al., 2011; Won et al., 2012). Above all, fruit juices and food-derived flavonoids have evoked tremendous interest, as reflected in myriad reports and reviews on this topic which often refer to results obtained from rodent and human studies (Farkas and Greenblatt, 2008; Fuhr, 1998; Mandlekar et al., 2006; Rodriguez-Fragoso et al., 2011; Won et al., 2010). However, clinical relevance is drug-dependent, and the metabolism model used is critical for extrapolation to human, since the amount, activity, substrate specificity and tissue distribution of metabolic enzymes considerably vary across species (Hurst et al., 2007; Komura and Iwaki, 2011; Martignoni et al., 2006; Tang and Prueksaritanont, 2010). For instance, intestinal metabolism has been reported to be remarkably more extensive in cynomolgus monkey than in human (Komura and Iwaki, 2011; Takahashi et al., 2009, 2010). Appreciable interspecies dissimilarities in metabolic fate have been shown for indinavir and atomoxetine, among others, contributing to up to 10-fold difference in oral bioavailability (Lin et al., 1996; Mattiuz et al., 2003). In addition, typically diminished metabolic reactions have been outlined for distinct species such as acetylation in dogs or sulfate conjugation in pigs (Martinez et al., 2002).

According to a recent review by Tang et al. (2010), multiple animal models can be used for CYP3A inhibition studies, whereas induction data may be better gained with rhesus monkeys, cynomolgus monkeys and beagle dogs, under the tacit assumption that standard inhibitors and inducers are involved (Tang and Prueksaritanont, 2010). The porcine model has been excluded from most of the reviews evaluating animal models for predicting drug metabolism (Martignoni et al., 2006; Tang and Prueksaritanont, 2010). Porcine CYP3A29 is known to exhibit comparably high protein similarity to human CYP3A4 (Suenderhauf and Parrott, 2013) and the pig has been sporadically used for assessing drug–nutrient interactions at the metabolic level (Wein et al., 2012). There is a need for more detailed investigations on porcine drug-metabolizing enzymes, though (Puccinelli et al., 2011). The shortcomings of *in vivo* models to assess intestinal microflora metabolism as implicated by the development of a rat model with associated human colonic bacteria (Hurst et al., 2007) may be faced with the porcine model since the pig's colon is populated with bacterial microflora resembling human conditions (Martinez et al., 2002). However, the (intermediate) metabolites from fermented substrates produced by pig colon microbiota can be very different from that of human colon microbiota, indicating that the microbial composition on species/strain level is different between pigs and humans.

Especially for the pig which, being fed *ad libitum*, is sometimes used to model obesity, but for any *in vivo* metabolism model in general, diet restrictions and standardization are recommendable, since dietary habits can confound metabolic activities (Martinez et al., 2002; Suenderhauf and Parrott, 2013). In general, the rodent model appears to be less appropriate for compounds that undergo extensive intestinal metabolism, as indicated by qualitative and quantitative human-rodent discrepancies in the expression of major intestinal drug metabolizing enzymes (Cao et al., 2006; Komura and Iwaki, 2008; Tang and Prueksaritanont, 2010), even in transgenic rodents expressing human CYP3A4 (Lin, 2008). Irrespective of the animal model, knowledge about the absorption, disposition and metabolism profile of both drug and nutrient is essential for *in vivo–in vivo* extrapolation of food–drug interactions (Benet, 2009; Wu and Benet, 2005).

10.2.6. Transporter-mediated processes

Transporter-related nutrient–drug interactions can occur at any site with absorptive and extractive characteristics. Intestinal, hepatobiliary and renal carrier-mediated transport as well as transporter modulation at the blood–brain barrier have been of predominant concern throughout literature (Chandra and Brouwer, 2004; ITC, 2010; Lai, 2009; Tang and Prueksaritanont, 2010;

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Won et al., 2012; Xia et al., 2007). Food-induced modulation of transporter processes can therefore affect absorption, distribution, metabolism and excretion to different extents in dependence of the exposure to the causative ingredients at the various sites. Similar to metabolism-related interactions, fruits, vegetables, herbs and their secondary metabolites have drawn increasing attention with respect to modulation of transporters during the last two decades, starting with the intensively described inhibition of P-gp and CYP 3A4 by grapefruit juice and gradually widening out to specific phytochemical-based alteration of both influx and efflux processes (Bailey, 2010; Deferme and Augustijns, 2003; Rodriguez-Fragoso et al., 2011; Won et al., 2012; Zhang et al., 2009). Interestingly, lipids and bile salts can also contribute to a decrease of transporter function in the postprandial intestine, implicating that not only specific food products and phytochemicals derived therefrom can affect transporter-mediated drug absorption, but also the ingestion of a fat-containing meal in general (Custodio et al., 2008).

Focusing on intestinal absorption, perfusion studies in rodents appear to be advantageous for elucidating mechanisms and regional characteristics of interactions, allowing simultaneous and localized assessment of intestinal permeability, metabolism and transport (Deferme et al., 2002; Lennernäs, 2007a; Shirasaka et al., 2010). Excluding the poor correlation with respect to intestinal metabolism and oral bioavailability, Cao et al. (2006) depicted reasonable similarities between rat and human regarding drug intestinal absorption profiles and expression pattern of PepT1, SGLT-1, GLUT5 and MRP2 in the small intestine (Cao et al., 2006). Detailed reviews of *in vitro* and *in vivo* models used for the evaluation of transporter-mediated interactions have been published (ITC, 2010; Xia et al., 2007). In summary, comprehensive investigations of transporter expression and activities in large animal models such as pig, dog and monkey are requisite to establish better *in vivo* surrogates of transporter-related interaction studies in human. Moreover, selective inhibitors or antibodies have not yet been identified for most transporters, especially with respect to drug uptake pathways (Xia et al., 2007).

10.3. Conclusions on food effects and in vivo model selection

It is evident that well defined procedures are required to encompass the variety of potential drug–nutrient interactions to ensure therapeutic efficacy in clinical practice. For this purpose, *in vivo* models are indispensable tools since the impact of food digestion is best assessed in intact animals expressing dynamic responses in drug absorption, distribution, metabolism and elimination rates in accordance to the complexity of physiological reactions to food intake in human.

Animal models usually require highly sophisticated approaches comprising varied types of formulation, administration sites and probe substrates with defined sampling schemes and surgical interventions. These efforts allow the estimation of the extent to which food and food-derived components affect drug exposure in plasma and specific tissues. Combination with *in vivo* imaging techniques, *ex vivo* and post mortem investigations is recommendable for an improved understanding of the relevance of food effect in oral drug bioavailability. Since the mechanisms of drug absorption and distribution often differ across species, comprehensive knowledge about model-specific pathways is necessary. Especially for large laboratory animals such as pigs, dogs and monkeys, qualitative and quantitative information about transporter and enzyme expression and function is poor.

Overall, the dog is the most studied *in vivo* model to evaluate common food effects on drug absorption due to physiological and dosage form related advantages as well as the ability to consume large infrequent meals on command. For mechanistic studies and investigations requiring extensive surgical or genetic interventions, rodent models are preferred as in case of permeability, lymphatic uptake or transporter-related interaction studies. Pigs and monkeys are alternative preclinical surrogates which require more validated approaches in assessing drug bioavailability in the fed state. The monkey seems to bear potential for overcoming physiological limitations of the canine model. Irrespective of the animal model, intra-species differences in the postprandial state related to sex, age, strain, diet and housing conditions need to be assessed systematically in future work for estimating the robustness of each model and establishing adequate ranges of study parameters.

Adaptation of the study performance might be considered to yield GI responses which best resemble human postprandial conditions. For example by pharmacological stimulation of gastric acid secretion and gastric emptying time controlling in the dog or diet restriction in the pig (Lentz et al., 2007; Suenderhauf and Parrott, 2013; Yamada et al., 1995). Of course, each additional modification needs to be validated carefully and progressive use of standardized techniques is necessary to confirm the benefits and uncover systematic limitations, respectively. With regard to the standard meals recommended by the regulators for human studies, there is an urgent need to define specific meal compositions to be applied in fed animal studies, as the common practice ranges between liquid diet mixtures, standard or enriched animal and human diet of various quantities (Kondo et al., 2003b; Lentz, 2008). Looking beyond acute responses to meal digestion, well defined feeding experiments can provide interesting insights into the effect of dietary habits on drug absorption, distribution and metabolism. This aspect has been accounted for in the draft guidance on drug interaction studies released by the FDA in 2012, recommending that uncontrolled consumption of dietary/nutritional supplements and distinct food products or beverages containing alcohol, grapefruit, apple, orange, vegetable from the mustard green family, chargrilled meat and tobacco should be avoided for 1 week prior to the start of the interaction study until its conclusion (FDA, 2012). Focusing further on possible effects of distinct food components on drug bioavailability, determining food-derived causative ingredients and exploring their pharmacokinetic properties as well as (multiple) interaction mechanisms across species are challenges yet to be faced. Eventually, the evaluation of the BCS and BDDCS in common laboratory species revealing interspecies classification differences and similarities might be a reasonable approach beneficial to both veterinary and human pharmaceutical research.

11. *In vitro in vivo* correlations

It is necessary to understand how changes during development to formulation and/or manufacturing process affect *in vivo* performance, and thereby safety and efficacy. This is also a central aspect to ensure that batches produced during routine manufacture will continue to give consistent local and/or systemic exposures to those evaluated in the pivotal clinical studies. As it is not practical to measure the PK of every batch of drug product in man, some of this understanding and verification must be based on *in vitro* testing. The development of IVIVC or *in vivo in vitro* relationship (IVIVR) is a key topic in all drug development programs and submission for marketing approvals, as this is the basis for understanding how product performance measured *in vitro* is likely to relate to performance *in vivo*. The gaps and issues in developing IVIVC or IVIVR for orally administered drug products are discussed from an industrial and regulatory perspective.

11.1. Definitions

An IVIVC is traditionally defined as a predictive mathematical relationship between *in vitro* dissolution and some aspect of

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in vivo exposure, covering either the entire absorption curve (Level A) or an individual parameter associated with the rate or extent of absorption (as in Level C correlations). However, in literature and practice the term IVIVC is often used in a more holistic sense, to describe a wide range of approaches linking some aspect of *in vitro* formulation behavior to the measured or predicted clinical performance of dosage forms. Indeed, the BCS system represents an example of a different kind of IVIVR, as it defines when changes in *in vitro* dissolution will have no impact on BE, for specific compound types and test conditions. This thinking is effectively the basis of biowaivers for BCS Class I (and BCS class III within EU) compounds. Stimulated by the advent of QbD, this thinking has recently evolved further into the 'safe space' concept, in which a risk-based approach is applied to determine the range over which dissolution may vary without altering bioavailability, for a specific drug product and API. This concept has been proposed as a basis for setting *in vivo* relevant *in vitro* specifications in the same manner as a traditional IVIVC.

There are three possible relationships between *in vitro* dissolution and *in vivo* performance, as described by Dickinson et al. (2008).

1. A mathematical correlation between *in vitro* dissolution and *in vivo* performance, such that a given change in *in vitro* dissolution can be used to predict the corresponding change in an *in vivo* exposure parameter (e.g. area under the curve (AUC) or maximum concentration), i.e. a classical IVIVC.
2. Changes in *in vitro* dissolution can be tolerated without any impact on *in vivo* performance, resulting in a dissolution 'safe space'.
3. Small changes in *in vitro* dissolution performance have no impact on *in vivo* exposures, but larger changes do.

For the purposes of this article, the term 'IVIVR' will be used to refer to scenarios 2 and 3 above, i.e., relationships other than a classical IVIVC, developed using both *in vivo* and *in vitro* data, that enables the impact of a given *in vitro* dissolution profile on *in vivo* performance to be understood. This is sometimes described by other authors as a nonlinear IVIVC (e.g., Polli, 2000) (this is not the same as a mathematical nonlinear correlation e.g., a nonlinear Level A) (Polli, 2000). It should be noted that these three scenarios are part of a continuum – ultimately when dissolution is slowed beyond a certain rate it will begin to impact *in vivo* exposures. The relationship detected in an *in vivo* study depends on where in the dissolution space the profiles to be tested lie. All three scenarios can be used to understand the relevance of a particular *in vitro* test result for *in vivo* performance. Scenarios 1 and 3, i.e., where at least one of the profiles tested produces a change in exposures, are perceived to offer a greater degree of control, as the detectability of an *in vivo* 'failure' provides assurance of the relevance of the dissolution test. However, for routine manufacture Scenario 2 offers a greater degree of assurance, as it demonstrates that the product lies within a 'safe space' where changes in *in vitro* performance will not be reflected *in vivo*.

11.2. Purposes of IVIVC/IVIVR in drug development

The purpose and application of IVIVC/R will evolve as a drug progresses through development. In the early phases, the focus is likely to be on establishing understanding of the potential clinical impact of formulation switches and changes made during manufacturing process development. At this stage, IVIVC/R tends to be drawn from across several data sources (e.g., performance of simple formulations in SAD/MAD studies vs. early solid dosage form prototypes, data form preclinical studies, TNO-TIM1) to create a holistic picture of the likely impact of a given change in man. At later stages of development (i.e., during and after the pivotal clinical safety and efficacy studies), IVIVC/R is used to provide regulatory evidence of relative bioavailability or BE to support formulation and process changes, definition of manufacturing ranges or design space. Here, the evidence to support IVIVC/R is more likely to be drawn from a single appropriately powered human bioavailability study performed specifically for this purpose. At the time of product registration, the understanding gained from IVIVC/R is also useful to link the proposed *in vitro* quality control release methods and acceptance criteria to *in vivo* performance registration, to give assurance that batches produced during routine commercial manufacture will be of appropriate clinical quality (i.e. be bioequivalent to batches used in the pivotal safety and efficacy studies). From a regulatory perspective, it is of course desirable to have a dissolution test and acceptance criterion which are not only discriminating between batches of different quality, but are also relevant for *in vivo* performance. However, while the guidances from FDA and EMA allows for biowaivers based on IVIVC or BCS, there is currently no regulatory guidance which mentions the use of other forms of IVIVR in this context.

11.3. Learning from previous reviews of IVIVC/IVIVR

The gaps and limitations in the practice of IVIVC and IVIVR have previously been discussed by other authors. These reviews have mainly focused on modified release MR formulations, which represent a high proportion of IVIVC studies described in the literature. This is unsurprising, as this formulation type is *a priori* more likely to produce IVIVC, having been purposefully designed so that dissolution will become rate limiting for the overall absorption. The main points from each of these reviews which are pertinent to the topic under discussion are recapped below.

Dokoumetzidis and Macheras (2008) ascribe failure of IVIVC for MR products to an inability to adequately reproduce or simulate the complexity of the holistic *in vivo* environment using current *in vitro* or *in silico* techniques (Dokoumetzidis and Macheras, 2008). Specific examples of this cited by the authors include:

- Inability to adequately replicate the composition of the luminal media in which *in vivo* dissolution takes place, including changes in composition along the intestine (which can potentially bring about an interplay between hydrodynamics, luminal fluid composition and dissolution behavior).
- Under-stirring and heterogeneity of mixing meaning that what appears to be a well-controlled and reproducible dissolution behavior *in vitro* is not the case *in vivo*.
- Inability to adequately simulate intestinal hydrodynamics and flow, which may lead to a discrepancy between *in vitro* and *in vivo* results, or induce such a high degree of variability for drug products which are sensitive to these factors that IVIVC is not shown.
- Complex dynamic interplay between several characteristics of the *in vivo* environment which are important for dissolution, which cannot be adequately captured by reproducing these individual factors *in vitro* or *in silico*.

Cardot and Davit (2012) described some of the limitations of performing mathematical IVIVC for MR and IR formulations (Cardot and Davit, 2012). The authors describe several aspects of the data manipulation step which must be carefully considered to maximize the likelihood of successful IVIVC:

- The use of mean vs. individual *in vivo* curves.
- Whether it is appropriate to correct for a lag time or apply time scaling.

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- The potential confounding effects of 'flip-flop' kinetics (where absorption rate is significantly slower than elimination rate), or using data from formulations with different bioavailabilities.
- Differences between subjects used to build the original IVIVC and those used to validate its predictive ability.

The authors describe a potential error in correcting for lag time when this is caused by gastric emptying, stating that this is not reproducible between subjects and so should not be corrected for. They also raise the issue that an IVIVC for highly variable drugs is problematic, as within-subject variability may mask formulation differences. Taken together, these two points highlight an interesting assumption inherent in the *in vivo* component of IVIVC/R studies, namely that the *in vivo* factors which govern dissolution of the dosage form are so reproducible between individuals that they do not affect the outcome of the study. In addition, variability in the *in vivo* factors governing dissolution can have an impact on whether an IVIVC is likely to be successful. This emphasizes a gap in current practices of clinical IVIVC studies – when performing *in vitro* dissolution measurements we need to routinely monitor and control aspects of the system to ensure that differences in the testing apparatus do not confound our ability to measure the performance of the dosage form. However, no such characterization of the *in vivo* system can be performed, despite the fact that the scope for variation between test systems here is far greater. Characterization of parameters such as gastric emptying time, local pH or even pressure forces prevalent in the GI environment could be performed in a relatively simple and non-invasive manner. The collection of such data on each individual dosing occasion may enhance our interpretation of the PK and increase the likelihood of building a quality IVIVR.

Jiang et al. (2011) discussed the use of physiologically based biopharmaceutical modeling in drug development (establishing IVIVC) from a regulatory perspective (Jiang et al., 2011). This modeling is a valuable tool in the development and regulatory environments, in particular providing opportunities for exploring BE of complex drug products and in the QbD environment, and encourage drug companies to explore its application. However, they express concerns over the 'black box' nature of many *in silico* models, and suggest that the same scientific question should be assessed using more than one modeling software package to ensure that consistent results are obtained. Additionally, a need for standard modeling study designs and acceptance criteria is identified.

Polli (2000) states that a Level A IVIVC is the least likely outcome for IR products, as they tend not to have dissolution-rate limited absorption (Polli, 2000). However, nonlinear forms of IVIVC, (i.e., where the plot of fraction dose absorbed vs. fraction dissolved is non-linear, with dissolution occurring faster than absorption) are applicable and useful. Polli argues that the term 'IVIVR' is preferable to 'IVIVC', to remove the implication that a study has failed if a linear mathematical relationship is not developed. To maximize the benefits of IVIVR in drug development and in the regulatory context, a better understanding of both *in vivo* dissolution and the *in vitro* dissolution test is needed.

11.4. IVIVC for IR products

In general, the topic of IVIVC and IVIVR for IR products has not received the same level of attention in the literature as IVIVC for MR products, but there exist a number of reports where IVIVC has been achieved. For instance, a Level C IVIVC was developed for four marketed carbamazepine IR tablets (Lake et al., 1999). The *in vitro* data from two simple pharmacopoeial-type dissolution tests is related to their relative bioavailability in man, and used to calculate a release specification which will assure formulation BE. Rouini et al. (2008) reported a similar example for five marketed gemfibrozil formulations, using dissolution tests in simple pharmacopoeial apparatus (Rouini et al., 2008). Kovacevic et al. (2009) report Level A IVIVC for carbamazepine developed across both IR and MR formulations, again relating to a simple pharmacopoeial dissolution test and media (Kovacevic et al., 2009).

For other compounds, use of more complex *in vitro* systems has enabled an IVIVC to be developed. Buch et al. (2009) describe the use of a combined dissolution and permeation system, to predict the *in vivo* performance of fenofibrate (Buch et al., 2009). Six different fenofibrate formulations were tested in this system using biorelevant dissolution media, and the *in vitro* data obtained was shown to correlate with exposure in the rat. In a later study, it was necessary to modify this system to predict the performance of formulations in man, to focus mainly on the effect of micellar entrapment by surfactant in the formulations on the permeation step (Buch et al., 2011). Dissolution testing in USP2/Paddle apparatus was not able to predict the *in vivo* performance of the formulations. Okumu et al. (2008) studied the dissolution behavior of montelukast sodium tablets in USP4/Flow-through cell apparatus, using biorelevant dissolution media and a dynamic pH change protocol (Okumu et al., 2008). By using the dissolution profile from this system in combination with Gastroplus® modeling, they were able to obtain a good fit for the *in vivo* plasma profile obtained from this formulation. Dissolution profiles from USP2/Paddle apparatus under various testing conditions were not able to model the clinical data as closely.

The examples above demonstrate that the use of more complex *in vitro* dissolution systems and protocols may increase the likelihood of a successful IVIVC, as they mimic the *in vivo* dissolution/absorption environment more closely than simple USP1/Basket or USP2/Paddle apparatus. However, while they are very useful to guide formulation and process development, these systems are not suitable for routine batch release testing. Simpler release tests more suitable for routine use would therefore need to be developed to utilize the full benefits of IVIVC during and after product registration. However, the mechanistic understanding gleaned from more complex *in vitro* systems may enable the rate controlling mechanisms for dissolution to be determined, so that a simpler test which reflects these can subsequently be developed.

The majority of reports in the literature where IVIVC has been attempted utilize BCS Class II compounds. Due to the lack of reporting of failed IVIVCs, it cannot be stated with certainty whether this is due to a lack of effort for drugs from other BCS classes, or whether a large number of examples of failed attempts for these classes exists which have not been reported. The former situation seems the more likely. The boundaries for 'high' solubility and permeability in the BCS system are set very conservatively, which is appropriate to their current use in defining an area of very low risk for biowaivers which encompasses any given formulation, drug substance properties, manufacturing process and manufacturing site.

However, in practicality this also means that, with the exception of BCS Class I, each BCS class will contain compounds with a broad spectrum of properties, and therefore potentially different rate limiting steps for absorption. For example, a low solubility compound which narrowly misses the 90% f_{abs} boundary (or 85% for EMA) would fall into BCS Class IV, and yet is very unlikely to have permeability-limited absorption – for such a compound, IVIVC is still likely to be possible irrespective of its BCS IV classification. Similarly, not all compounds in Class II should be expected to show IVIVR, an example of which is described below. The BCS as it currently stands is therefore an inadequate system to assess the likelihood of IVIVC for a particular compound.

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11.5. IVIVR for IR products

The implementation of QbD has lead to a renewed focus on the relationship between formulation and manufacturing process variables and *in vivo* performance during product development. This has led to the establishment of dissolution tests and release specifications based on clinical bioavailability data, which are used in defining the Design Space and are a key component of the Control Strategy. Dickinson et al. (2008) present a case study applying this approach to a BCS Class II compound (Dickinson et al., 2008). Tablet variants were manufactured based on the commercial formulation but incorporating the highest risk process and formulation dissolution failure modes, as determined by a product-specific Quality Risk Assessment. These tablet variants were then dosed in a clinical relative bioavailability study, and exposures compared to the standard tablet and an oral solution. The study showed that all of the tablet variants gave equivalent exposures to the standard tablet, despite having slower dissolution *in vitro*. Additionally, even the slowest dissolving tablet variant gave equivalent exposures to the oral solution. Thus, a 'safe space' for dissolution performance had been established, i.e. a range of dissolution profiles which would result in BE *in vivo*. This indicates that dissolution was not rate-limiting over the range of profiles tested. The authors hypothesized that this was due to sufficiently high solubility so that the dissolution was faster than other physiological processes (such as permeability and/or gastric emptying), despite that it is classified as a BCS Class II compound. The authors subsequently used the results of the clinical relative bioavailability study to select an appropriate dissolution test, and set a release specification which would assure BE was maintained. Similarly, Buggins et al. (2011) described a case study for a BCS Class IV compound, where three tablet variants with slowed *in vitro* dissolution profiles encompassing the highest risk failure modes for dissolution gave equivalent exposures to a standard tablet (Buggins et al., 2011). This was attributed to the fact that the compound had good permeability and high intestinal solubility, despite its BCS IV classification.

This approach is starting to gain acceptance from a regulatory perspective. In a recent FDA presentation, the 'safe space' concept was included as a method of setting dissolution specifications which are linked to clinical performance, which may lead to a wider dissolution specification being granted than if no data linking dissolution to *in vivo* performance had been generated (Pope Miksinski, 2011). However, the IVIVR/'safe space' concept is not currently included in any regulatory guidance documents, meaning it is not as well established from a regulatory perspective as traditional IVIVC. This is somewhat paradoxical, as it is more difficult to gain regulatory flexibility for a product where *in vivo* performance has been proven to be insensitive to dissolution changes, than for a product where any change in dissolution release rate results in measurable changes in the rate or extent of absorption. This may be because for IVIVC, the presence of a mathematical correlation can be perceived to give a greater assurance of control. Incorporation of *in silico* modeling approaches into the design and interpretation of 'safe space' studies may help to dispel this perception, for example by demonstrating that *in vivo* performance would not be expected to show sensitivity to dissolution over the range of profiles tested in the clinical study.

The examples above indicate that, for a well designed IR tablet, 'safe space' may be a more likely outcome than IVIVC. They also demonstrate that the use of BCS class alone can be misleading regarding the likelihood of IVIVC development. A more detailed understanding of the space over which formulation dissolution is truly rate limiting for a particular API from a particular formulation is needed. As well as enabling more efficient design of IVIVC/IVIVR studies and development programs from an industrial perspective, this would help set expectation regarding whether an IVIVC could truly be developed, or whether other outcomes such as 'safe space' are more likely, and may thus give additional confidence to regulators when these outcomes are achieved.

An interesting gap in the reported data in the literature is the absence of IVIVC or IVIVR studies in patient groups. Naturally, healthy volunteers are usually the dosing group of choice for relative bioavailability and BE studies, as the absence of the confounding effects of disease and co-medications on the absorptive environment should reduce the 'noise' and make them more discriminatory for detecting differences in formulation performance. However, for certain classes of compound (e.g., cytotoxics used in the oncology setting), studies in healthy volunteers are not permitted from a safety perspective. Additionally, it would be unethical to dose formulation variants which may have suboptimal performance to patients who are expecting to receive therapeutic benefit from the treatment. This raises the question of how best to link *in vitro* and *in vivo* performance for these compound types. During development, increased reliance is likely to be placed on pre-clinical studies, complex *in vitro* dissolution (e.g.TNO-TIM-1) and *in silico* modeling to support formulation bridges and design the dissolution release test. However, the absence of a robust way to prove that these models to adequately describe drug absorption in man for the compound in question limits the degree of reliance which can be placed on them. More work is needed to develop innovative ways of defining IVIVR for these compound types, for use both during development and in the regulatory BE environment.

11.6. Gaps in the current state of the art in IVIVC/R

While there are some interesting and innovative examples of the development and application of IVIVC and IVIVR in product development, further work is needed to increase the likelihood of developing a successful IVIVC/IVIVR, and enable them to be fully utilized in drug development and regulatory practice. Current gaps in the state of the art are summarized below.

11.6.1. General gaps

- Better understanding and mapping of the compound space over which dissolution is likely to be rate determining for *in vivo* exposures is needed. This would form the basis for intelligent design of IVIVC/R studies, and provide a platform of common understanding on which to base discussions with health authorities. The current BCS system does not adequately fulfil this, and can create false expectation as to the likelihood and risk associated with IVIVR in the minds of scientists and health authority reviewers.
- More examples of IVIVR are needed in healthy volunteers and patients, to enable success factors and limitations to be better understood.
- More examples of the use of *in silico* simulations as part of IVIVC/R, and an understanding of how to use these in a regulatory context.
- Better knowledge is needed about the acceptable use of IVIVR and the 'safe space' concept in the regulatory environment, as this is currently not described in any of the BE guidelines. The current guidances allow for biowaivers based on IVIVC or BCS Class I and III 'safe spaces', but do not allow for compound-specific 'safe spaces'.
- More examples of the use of IVIVR as part of an overall QbD strategy are needed, including examples of selection of tablet variants on the basis of highest product specific risk mechanisms. Also, better understanding of the use of such IVIVRs in the regulatory context is required.

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11.6.2. In vitro systems

- Difficulty in mimicking the complex and dynamic environment for *in vivo* dissolution *in vitro*.
- Difficulty in translating the understanding gained form complex *in vitro* apparatus into a simple pharmacopoeial-type test and acceptance criterion.

11.6.3. Difficult compound types

- Compounds where human studies cannot be performed in healthy volunteers (e.g. oncology) – how do we generate understanding for these compounds to support relevant dissolution tests and specifications?
- Highly variable drugs – how do we demonstrate IVIVR for these compounds without dissolution changes being swamped by *in vivo* variability?
- Failed attempts at IVIVC/R are not generally reported, making it difficult to fully define which other types of compound are problematic.

11.6.4. Use of innovative clinical study designs

- *In vivo* factors impacting dissolution are not routinely characterized in IVIVR studies, however this would enrich the information gained and aid data interpretation.
- Adaptive study designs appear to be under-utilized in IVIVR. This approach is likely to increase the efficiency of IVIVR studies, reducing the number of testing arms by allowing the study either to stop once the 'safe space' region is reached, or allowing specific mechanisms to be probed based on feedback from the initial study arms.

11.7. Conclusion regarding IVIVR/IVIVC

Developing an understanding of the link between *in vitro* performance and clinical exposures is of critical importance for all drug products. This understanding is needed in order to streamline the development process, to understand the risk of a change in formulation or process significantly affecting bioavailability, and ultimately to provide assurance of consistent clinical quality of batches produced during routine manufacture. However, several gaps in the current state of the art impede the full and efficient utilization of IVIVC and IVIVR approaches. Working to address these will be of benefit to both drug developers and regulators, and ultimately to the patient.

12. Models for predicting API and API-formulation approaches and their correlation with *in vitro* and human

Processes for appropriately and accurately selecting formulation strategies to progress important drug candidates have a number of benefits. These include directing the right resources to the right problems at the right time with the right level of effort. Aligning formulation strategies that provide for adequate pharmaceutical and biopharmaceutical performance as well as clinical outcomes without using systems that exaggerate needs and costs are crucial to effective drug development. The earlier that these concerns can be addressed, the lower will be the need for rework with ensuring shorter cycle times, lower costs and potentially higher overall quality.

The purpose of this section is to present formulation selection philosophies focussing on preclinical *in vivo* tools. This section will also include a discussion on important drug candidate properties impinging on formulation selection such as API physicochemical properties, pharmaceutical manifestations and biopharmaceutical interactions as well as early approaches to frame formulation appropriateness as a function of two important drivers including the needs of the compound and the ability of a particular organization to address these needs into which category items such as downstream capabilities and capacity, cost of goods and related factors fall. Ultimately, deciding whether a simple formulation platform could be considered or if enabled strategies are required is the goal of these evaluation methodologies.

For discovery-based projects, the opportunity exists for an early assessment of drug characteristics and contribute to the compound selection and development (Ding et al., 2012; Ku, 2008; Li and Zhao, 2007; Maas et al., 2007; Saxena et al., 2009). These working models have been evolving over time in many organizations. This evolution relies on interconnectivity with upstream medicinal chemistry and biological assessments components. To this end, problematic compounds and projects are often identified at early stages allowing for drug-ability, formulate-ability and formulation process-ability assessments to be leveraged early in the development cycle. These evaluations which are part and parcel of the internal formulation decision tree will be outlines as a function of this section.

The general formalism of a formulation decision tree is rooted in a number of science-based, data-driven factors incorporated into pharmaceutical company practices that are refined by experience as well as the information from the external scientific community. The general strategy for selecting a formulation often follows the suggestion of Branchu et al. (2007) which divides the problems into three parts (Fig. 9) (Branchu et al., 2007):

1. What is the nature of the formulation challenge and are conventional or enabled strategies most appropriate for the selected API.
2. If enablement is needed which enabled system is most appropriate.
3. For a selected enabled formulation strategy, what are the most important design space considerations that can be suggested based on the nature of the data available.

These factors, by definition, heavily target "compound need" elements and how these are balanced with capacity, capability and costs represent important downstream considerations in bringing the successful product to the market.

A variety of factors may contribute to deciding whether formulation enablement will be likely needed. API properties are crucial to understanding how a compound should be formulated and these are derived from several important assessments associated with drug absorption. While there are many factors that impact the PK and PD aspects of a drug candidate, the most important feature include the exposure of the body to the drug candidates both in terms of its rate and extent of absorption. Thus for an IR solid oral dosage form (representing about 80% of current formulation development), it must disintegrate and dissolve releasing the API in a solubilized form. Solubility and permeability therefore form the basis for BCS which attempts to describe and categorize drugs based on their biopharmaceutical properties. The scale-up and post approval change (SUPAC) apply BCS in regulatory decision. These values and boundary conditions incorporated in the BCS are intended as guidelines for granting biowaivers for clinical studies intended to validate formulation changes post-approval meaning that the definitions are designed to be strict and specific to that purpose. While solubility and permeability are essential to understand when and how much support is needed to validating a formulation change, the underpinning scientific framework is also useful in assessing drug candidates in terms of their formulate-ability (Wei et al., 2008). This is done using a variant of the BCS, i.e. the developability classification system or DCS (Butler

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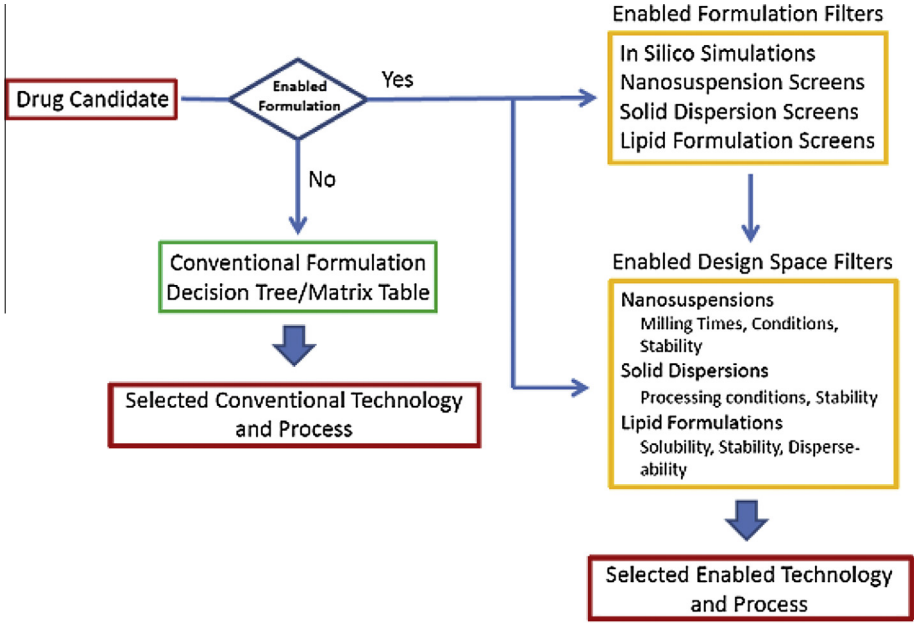

Fig. 9. Selection strategy for enabled (non-conventional) formulations.

and Dressman, 2010). This paradigm differs from BCS in that it focuses on the formulation rather than on the API and attempts to assess factors useful in judging the formulation difficulty which may be encountered. The DCS separates Class II candidates into two types: namely Class IIa, compounds which are dissolution-rate limited in terms of their oral bioavailability and Class IIb, compounds which are solubility limited. This difference can be appreciated by considering the Noyes–Whitney equation:

$$\frac{dC}{dt} = \frac{DA}{Vh(C_s - C_t)}$$

where dC/dt is the dissolution rate, A is the surface area of the drug, D is the diffusion coefficient of the drug, V is the system volume, h is the thickness of the unstirred water layer separating the drug and bulk media, C_s is the saturation solubility of the drug and C_t is the concentration of drug at time, t. In a formulation context, if reducing the particle size or increasing wettability (i.e., increasing A or decreasing h) leads to an increased oral bioavailability, then such API's could be considered as dissolution-rate limited. If these manipulations do not impact dissolution rate or oral bioavailability, the system could be considered solubility-limited. This latter situation suggests that either the drug form should be changed to increase drug absorption (i.e., the chemical potential of the API should be increased) or that the additives should be included to decrease the chemical potential of the drug in the dissolved state. Thus, both in the context of DCS assessments and more broadly, knowledge of whether the drug is dissolution-rate or solubility-limited in terms of its oral bioavailability sets the stage for assessing formulation complexity. That is, this simple system provides an interesting insight as to the how the oral bioavailability of a drug candidate is limited which, by inference, can suggest how the limitation is best addressed. Type I compounds are soluble and permeable meaning that only factors that impact their ability to reach absorption sites are of consequence (e.g., gastric emptying) making these systems relatively formulation independent. Class II compounds by contrast are limited in their absorption by their dissolution rate or solubility meaning that factors which enhance these system properties could be bioavailability-promoting.

12.1. Physicochemical API properties as indicators of oral delivery challenges

For poorly water-soluble compounds, the causes of their poor solubility can also aid in improving oral bioavailability through appropriate formulation concepts. This can be appreciated by assessing the empirical relationships described by Ran and Yalkowsky such as the relationship presented below:

$$\log S_w = 0.5 - \log P - 0.01(\mathrm{MP}\ (^{\circ}\mathrm{C}) - 25)$$

where the log of the water solubility is given as a function of the log$P$ and the melting point (MP) (Ran and Yalkowsky, 2001). In other words, compounds can be limited in their aqueous solubility by wettability factors or by crystal lattice forces. Knowing which component is more influential in limiting the water-solubility can be instructive in selecting a solubilization strategy. In addition, the complexity of finding a solution is suggested by the two limiting conditions wherein log P-limited water solubility is more easily addressed than melting point-limited solubility. When crystal lattice energy limits solubility, the drug is not only insoluble in water but in other solvents and carriers as well. Based on these two factors, "grease-balls" (high log P materials) may be more easily formulated using solubilizing strategies while "brick-dust" (high melt point compounds) might lend themselves better to particle size reduction.

Historically, many attempts have been made to use physicochemical properties to scout out formulation trajectories based on the discussions outlined in the last few paragraphs. These considerations together with permeability features were used to suggest not only if an enabling formulation approach is needed but also to give insight as to which systems might best add value. This has taken the form of the following "play ground" diagram (Fig. 10).

Assessing a large group of formulated drugs, Branchu et al. assessed which were formulated conventionally and which were formulated using enabled technologies and then deconvoluted the physicochemical properties of the API (Branchu et al., 2007). This retrospective analysis found that the two groups of compounds had different properties which could be generally described such that enabled formulation were needed if the logD_o

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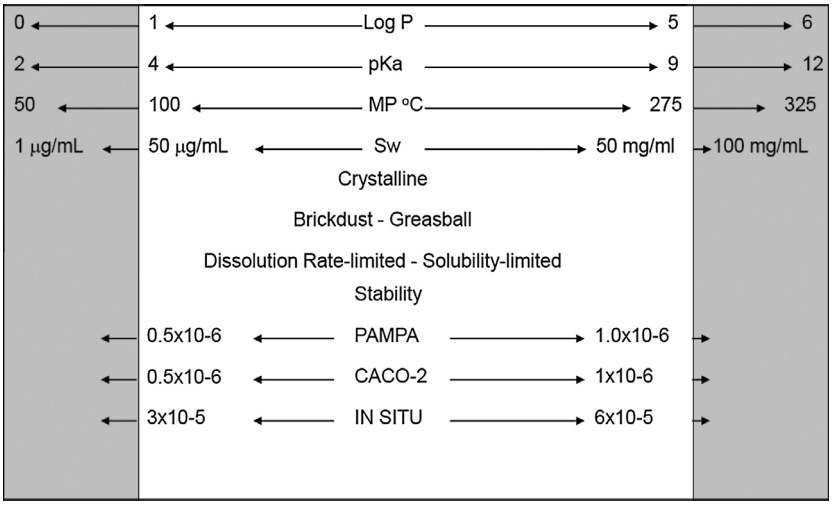

Fig. 10. Schematic view of solubility and derivative factors, including permeability, falling within conventional formulation space.

(Dose Number) < 1.7, log$D$ > 1.2, hydrogen-binding donors (HBD) = 0, hydrogen-binding acceptors (HBA) < 7 and molecule surface area >406 Å^2. The usefulness of this and related approaches has been limited when used prospectively with a number of root causes associated with the poor predictivity in the guise of both Type I and II errors. One of the most glaring is related to the biorelevance of these measurements. Thermodynamic solubilities, for example, of drug candidates in compendial buffers under carefully controlled conditions have the potential of dramatically over- or underestimating the solubility of that drug material in the body not only as a function of the dissolving media but also by inappropriately considering supersaturation and absorption.

12.2. Solution versus suspension dosing comparison as in vivo formulation finding strategy

Intravenous drug testing is often important for deriving parameters such as intrinsic clearance and other factors associated with the interaction of the drug with the biological systems (Balani et al., 2005; Neervannan, 2006), however these screening vectors do not provide insight as to whether a solid dosage form might be useful. To this latter end, Mackie et al., described a simple solution–suspension–intravenous comparison (Fig. 11) of drug candidates in test animals, most usually the rat and dog (Mackie et al., 2008). In this companion assay to API property and pharmaceutical assessments, a simple drug solution (generally as aqueous 20% w/v 2-hydroxypropyl-β-cyclodextrin (HPβCD)) is compared with a drug suspension (usually as 0.5% Methocel®, processed using a Covaris® homogenizer). The dose for both formulations is 10 mg/kg in the rat and 5 mg/kg in the dog. Blood levels are then determined using appropriate LC/MS-based techniques. This simple dosing design then generates a 2 × 2 matrix which can suggest a formulation trajectory along with other experimental and theoretical findings.

Solution/Suspension Equivalent High BA BCS I (or BCS I-like)	Solution/Suspension Equivalent Low BA BCS III (or high first pass)
Solution/Suspension Inequivalent High/Low BA BCS II (likely difficult)	Solution/Suspension Inequivalent Low/High BA Multiple Causes

Fig. 11. Solution–suspension–intravenous outcomes in a test species and their alignment with BCS.

If a solution and suspension give equivalent exposure, this suggests that the *in vivo* dissolution rate of the suspension in no way limits oral bioavailability. This suggests that the impact of a formulation on the ability of the API to be absorbed is relatively low. For cases where solution and suspension give similar exposure and the absolute oral bioavailability is high, a BCS I or I-like system is suggested. If on the other hand, a mismatch of expected maximum bioavailability (based on intravenous clearance) and observed bioavailability (lower than expected) occurs then this indicates permeability issues or precipitation within the GI tract suggesting a BCS III or III-like system or that the API is subject of high first-pass effects. Again for both of these systems, conventional formulation strategies would seem to be appropriate. For the situation where a solution gives significantly higher exposure than the suspension, the data suggests that *in vivo* dissolution rate or solubility is limiting, pointing to a BCS II material and one likely in need of enablement (i.e., a BCS II-complex compound). The fourth possibility is one in which a suspension provides for better oral bioavailability than a solution. Several root causes might be ascribed to this behavior including poor chemical stability of the API when in solution within the stomach environment, saturation of an uptake transport mechanism or initial supersaturation followed by precipitation of the API from the solution into a more poorly dissolving form than that associated with the suspension. If acid instability is assigned as the root cause, a number of formulation design elements can be considered such as enteric coating or co-administration of the drug with an antacid or proton pump inhibitor, the latter under the assumption that no interactions occur. Thus, the rat and dog are used as bioreactors to assess solubility, dissolution rate and permeability.

This approach has now been applied to more than 100 compounds (Mackie et al., 2012). Based on this perspective, a number of points have been made: A suitable suspension could be prepared for the vast majority of compounds using 0.5% w/v Methocel. In two situations, a slight modification was needed such that Methocel with Tween 20 was optimal for one compound and, in a second case, where HPβCD and Tween 20 were the best suspending agents. Drug solutions were possible using 20% HPβCD in 87% of cases. Other systems that were assessed included water for injection (1%), citrate–phosphate buffer (1%), TPGS in oleic acid (1%), SBEβCD (5%) and PEG400 (6%). Use of PEG 400 or others however

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presents a difficult situation as PEG has influences on motility and water flux in animals and humans to varying extents (Schulze et al., 2003). It should be used cautiously or in low concentrations. The 2 × 2 matrix could be bucketed using several classification cut-offs with AUC ratios of solution–suspension dosing <0.8 (suspension more bioavailable than solution), 0.8–1.2 (solution and suspension gave equivalent bioavailabilities), 1.2–2 (solutions were modestly better than suspensions) and >2 (solutions were significantly better than suspensions). The data suggested that: 18% of compounds demonstrated better bioavailability from a suspension than a solution, 45% of compounds demonstrated equivalent exposure from a solution and a suspension, 26% of compounds were modestly more bioavailable from a solution than a suspension and 10% of compounds were significantly more bioavailable from a solution than a suspension. Application of traditional tools based on thermodynamic solubility, log *P*, pK_a, intrinsic dissolution rate, PAMPA (parallel artificial membrane permeability assay) and related assays suggested that approximately 65% of the compounds would require some type of enablement to generate a useful form preclinically or clinically. That suggests that using only physicochemical properties may overdiscriminate the need for enabled systems with the application of technologies of higher complexities and costs than needed as well as a higher risk of rework. This combination of API, pharmaceutical and biopharmaceutical data then suggest whether formulation enablement is likely needed.

A similar suspension vs. solution approach has been performed by Muenster et al. using a physicochemical diverse Bayer pipeline compound set (Muenster et al., 2011). Here, a correlation of *in vivo* dissolution in the rat at respective predicted therapeutic doses in humans vs. actual *in vivo* dissolution data in humans (tablet or suspension vs. solution) of the same compounds was established. Data suggest that if AUC_{norm} suspension vs. AUC_{norm} solution in the rat is >50%, *in vivo* dissolution in humans is sufficient for the development of a standard IR tablet, without enabling technologies needed. Furthermore, correlation of suspension vs. solution in rat vs. dose/solubility ratios at various pH revealed a good correlation at pH 4.5 and 7, indicating that at dose/solubility ratios of <100 mL/kg (pH = 4.5) and <500 mL/kg (pH = 7) no enabling formulation technology is needed for the development of an oral market formulation for humans. However, a poor correlation between *in vivo* dissolution in the rat and dose/solubility ratios at pH = 1 was observed, suggesting that the rat is good predictor for neutral and acidic API dissolution, but may underestimate *in vivo* dissolution of weak bases in humans.

Early formulation finding strategies have also been described by Maas et al. who also assessed API, formulation and biopharmaceutical factors (Maas et al., 2007). In their approach, a number of formulation platforms are identified to address both simple-to-formulation API's as well as those in need of enablement. Intravenous dosing is first completed to verify that PK properties are appropriate and then is an oral suspension dosed, first in rat and subsequently in dog. If exposure is satisfactory, formulation trajectories are selected from the conventional toolbox. If suspension dosing provides for poor exposure, other manipulations of the API are considered including milling. These studies then chart a way forward for the possible formulation possibilities. Saxena et al. used a similar strategy with a comparison of drug solution and suspensions (Saxena et al., 2009). This decision tree thus suggested that bioavailability from a solution should be reasonable. If exposure of the suspension was more than 2-fold lower, a number of alternative processing technologies were suggested including salt screening, solid dispersions, lipid based methods and suspension/nanosuspensions. The approach suggested that the preclinical formulations should result in certain minimal pharmacokinetic properties to justify progressing the compound including an acceptable terminal half-life, a total clearance less than the hepatic blood flow and a bioavailability from the selected formulation of >30%. Li and Zhao (2007) suggested a similar approach with a comparison of solutions and suspensions to suggest whether simple or complex formulation would likely be of benefit (Li and Zhao, 2007). Zheng et al. (2012) suggested assigning formulation risk based on *in vitro* solubility as well as *in vivo* drug levels in test species after oral dosing of solutions (Zheng et al., 2012). Low risk compounds were those generating useful blood levels after dosing with vehicles containing <30% of an organic modifier and high risk systems were those requiring higher levels of an organic modifier. The decision tree outlined suggested that low risk compounds could be formulated using salts or particle size reduction while high risk compounds would likely require enabled systems including amorphous solid dispersions, nanosuspensions or lipid-based strategies. Based on the possible complexity of high risk systems, additional work in parallel was suggested to optimize formulation finding.

12.3. Selection of the most appropriate formulation technology

The next step, after an assessment of formulation complexity is derived, is then to suggest which enabled technology is best placed to solve the specific issues associated with the drug candidates being considered (Kawakami, 2009). One approach is to extract value from various theoretical as well as down-scaled, automated filters to align a formulation type with the compound of interest (Fig. 12).

The philosophy associated with the formulation filters is based on a deconstruction of important formulation elements and then the development of tests to assess these aspects. Four possible enabled strategies are included in this process approach including nano-crystalline suspensions, amorphous solid dispersion, liquid-filled capsules and "others".

12.4. Inclusion of computational assessments as additional tools

Theoretical assessments are based on PBPK models with the two main tools including GastroPlus™, an advanced compartmental and transit (ACAT) model (SimulationsPlus, Lancaster, CA) and SimCyp, an advanced dissolution, absorption and metabolism (ADAM) model (Simcyp, Sheffield, UK). GastroPlus™ can be used both to predict API, pharmaceutical and biopharmaceutical properties based only on the chemical structure or with additional experimentally determined data with an increasing predictivity as a function of more and higher quality data (Hosea and Jones, 2013; Kuentz et al., 2006; Sjögren et al., 2013; Tsume et al., 2012). Thus, even with only the chemical structure, initial suggestions of the compound solubility and absorb-ability can be estimated and reported in terms of a dose, dissolution and absorption number (D_o, D_n and A_n, respectively) as well as the maximum absorbable dose (MAD). The terms are defined as follows:

- Dose number (D_o) – the dose divided by the product of delivered volume (250 mL) and solubility of the drug: $D_o = \text{Dose}/(V \times C_s)$ (the lower the better).
- Dissolution number (D_n) – the ratio of mean residence time and mean dissolution time (the higher the better).
- Absorption number (A_n) – the ratio of the mean residence time and mean absorption time (the higher the better).
- Maximum Absorbable Dose (MAD) – the product of the drug solubility, absorption rate constant, fluid volume and transit time (i.e., solubility and permeability are compensatory): $MAD = S \times K_a \times V \times T$.

An assessment of itraconazole (including API, pharmaceutical and biopharmaceutical properties) was completed using only the

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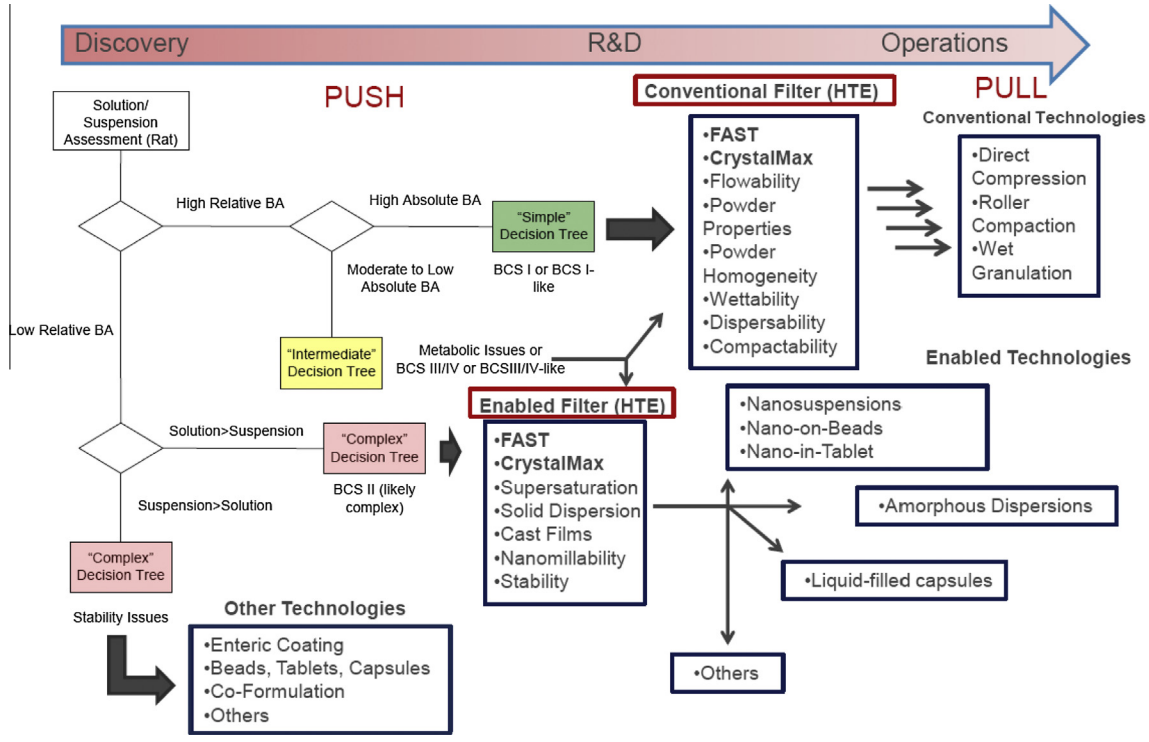

Fig. 12. Decision approach for the selection of an enabled technology.

chemical structure of the compound. This information-risk readout suggests that itraconazole is a BCS Type II/DCS type IIb drug with a low predicted water-solubility (~0.0006 mg/mL) and a MAD of 48 mg. This is in contrast to doses that are needed to generate a useful anti-fungal effect (200 mg loading dose and 100 mg maintained dose). A parameter sensitivity plot can also be rendered that suggests what the effect of drug solubilization might be on the f_{abs} (in this case the amount of drug reaching the portal blood) (Brewster et al., 2007).

These calculations suggest that the low f_{abs} is strongly influenced by the degree of drug solubilization and that even modest increases in this factor can strongly influence the amount of drug taken up into the presystemic circulation based on high intrinsic permeability. This analysis suggests that increasing the drug solubility from 0.0006 mg/mL to 0.1 mg/mL would make drug absorption almost quantitative. This information may then initiate an assessment of solubilizing technologies to generate an appropriate liquid or solid dosage forms. In the case of itraconazole, two useful solubilizing vehicles were identified including PEG400 (solubility of itraconazole = 2 mg/mL) and 40% w/v HPβCD (solubility of itraconazole = 10 mg/mL). GastroPlus™ suggested that both of these systems would increase the f_{abs} and this was verified in both animal and clinical studies. The HPβCD vehicle served as the basis for a marketed oral solution and intravenous formulation. Another use of the parameter sensitivity (spider plot) is to assess the possible application of an amorphous dosage form concept. In assessing this trajectory, an estimate is made of the solubility enhancing effect of converting the drug from its crystalline form to the amorphous phase. A variety of approaches can be assessed. The formalism of Hancock and Parks (2000) can be applied wherein the increased solubility is inferred using thermo-analytical data obtained by Differential Scanning Calorimetry (DSC) including the melting point and heat of fusion of the crystalline phase, the glass transition temperature and change in heat capacity at the glass transition temperature (T_g) for the amorphous phase (Hancock and Parks, 2000). In the case of itraconazole, the solubility ratio of amorphous/crystalline drug is 100. Assessing these data and increasing the drug solubility from 0.0006 mg/mL to 0.06 mg/mL would increase the drug f_{abs} to >75%. Clearly, these are gross estimates that do not take into account a number of important processes and factors but these early assessments can give some insight within a specified error as to whether a particular formulation direction is possible or less interesting.

In addition to solubilizing strategies, PBPK modeling can also shed light on the possible application of particle size reduction with regard to improved oral bioavailability. This is again completed using a parameter sensitivity analysis but in this case the variable is API particle size. That is, the percent drug absorbed (into the portal circulation) is assessed as a function of reducing the particle size into the nano-domain. Data thus derived suggest that, in this case, reducing the particle size even to 1 nm would not influence the oral bioavailability. This finding has since been corroborated in animal studies suggesting that the solubility of the compound is so low that particle size reduction techniques are not useful or that the saturation solubility is reached faster than disappearance of dissolved API is happening by diffusion/permeability.

Theoretical assessments (which are or are not bolstered by other data) are useful inputs but cannot fully position or align a formulation approach and compound. The formulation process is based on several factors including the need for processing information as well as data on the excipient design space, etc. To fill these knowledge gaps, experimental work is suggested. In order to complete these experiments in a time- and compound-efficient manner, down-scaled, automated tests have been designed and evolved to assess not only the most appropriate technology for the particular API but also to give insight into processing aspects of formulation. These miniaturized, automated workflows are designed to address key formulation questions to help identify the most appropriate dosage form platform, enabled strategy or direction as well as to eliminate possibilities that are not likely to add value. Implicit in all of these simplified models is that their output needs to be continuously confirmed and checked as a function of compound development.

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Key questions are therefore collected as a function of the four formulation directions. For a nanosuspension to be useful, the ability to mill an API to a desired size is important (mill-ability) as is the physical stability of the milled dispersion. For an amorphous solid dispersion to be useful, glassy carriers that adequately disperse the amorphous drug are needed and the resulting systems need to be stable to phase separation and crystallization. Finally, liquid-filled capsule strategies need to contain excipients that adequately solubilize the drug dose in an appropriate volume and the said dispersion needs to be stable both chemically and physically. Thus once enablement is tangibly decided upon, all three enabled possibilities are assessed using both fast and compound-sparing experimental approaches with a selection based on the assigned inclusion and exclusion criteria.

12.5. Nanosuspensions feasibility: mill-ability and dispersion stability

The workflow identified to assess the likelihood that a nanosuspension might serve as the enabled formulation strategy, are based on several purpose-built and generic automation platforms including a multiplexed nanomill. This attrition mill consists of 10 independent milling heads that can be independently controlled both in terms of milling speed and time. The systems makes use of 2.8 mL disposable milling chambers which can be filled using automated workflows and each milling station is actively cooled. Milling media are generally 0.5 mm highly reticulated polystyrene beads and particle size analysis is completed by harvesting the nanosuspension using an insulin syringe (U-100, 0.5 mL, 0.33 mm × 12.7 mm) followed by the particle size distribution assessment measured using a Malvern Mastersizer coupled with a Hydro μP dispersant unit and a Wyatt DLS plate reader.

The multiplexed mill is designed to assess multiple formulation aspects and design space elements in a concerted fashion to rapidly find fit-for-purpose formulations. In addition, the mill is configured to scale to larger processing situations (i.e., to the nanomill, dynomill and Netzsch mill). The experimental workflows generally begin with an assessment of excipient space (including primary and secondary stabilizers) and processing space (including milling speed and time). The important endpoint derived from these experiments includes mill-ability (the ability to reduce the particle size to a proscribed average and distribution) and dispersion stability over time and under various conditions.

A practical example may be useful in outlining the utility of this approach. Itraconazole was evaluated as an injectable nanosuspension in several clinical studies (Mouton et al., 2006). In developing this formulation, two key factors were screened as a function of development including which surfactant manifested the best milling and stabilizer properties and what was the optimal milling time. Both of these could be assessed in the downscaled multiplexed mill. Itraconazole was milled in the presence of several potential ionic and non-ionic stabilizers at ratios of 1:4 relative to the API. Standard protocols included milling the material for 60 min at 4000 rpm in the presence of 0.5 mm highly reticulated polystyrene milling media. Particle sizes were then assessed at the end of milling as well as at one and two weeks after milling with the samples stored at various conditions (5, 25 and 40 °C). Of the 25 unitary or binary stabilizer systems assessed, Poloxamer 388 proved to be the most useful under these conditions. Based on this excipient, milling curves were generated wherein itraconazole was milled for times varying between 15 and 300 min with physical stability follow-up. Based on d90 and d99 measurements, a suspension of a useful size could be generated after 120 min of milling, less time generated undermilled systems. At long milling times (i.e. 300 min), particle growth on storage was noted suggested that these systems were overmilled. Conditions from the downscaled multiplexed mill could be scaled to the Netzsch mill.

12.6. Solid dispersion feasibility

Important elements in the construction of a useful amorphous solid dispersion include the ability to dissolve or disperse a drug in a glassy or semi-crystalline polymer as well as the stability of the formed dispersion. As a consequence, amorphous solid dispersions should act as supersaturating drug delivery systems. That is, the release of the amorphous API, the rate of which is ideally governed by the dissolution of the glassy carrier, is such that nucleation and crystal growth is delayed and the formed supersaturated system is produced in a way to allow for sufficient drug absorption. Thus, excipients that act as nucleation or crystal growth inhibitors can increase the efficacy of the formulation. In the best case, the precipitation inhibitor is the glassy carrier. Alternatively, precipitation inhibitors can be added to the glassy carrier. Finding components that may impact supersaturation stability is therefore integral to building up a useful solid dispersion (Bevernage et al., 2012; Brouwers et al., 2009; Takano et al., 2010). Specialized workflows have been developed to screen for these and other properties. Specific tools to assess precipitation inhibition, film casting and down-scaled processing are available and these will be discussed in turn. Assessing whether excipients are available to impact precipitation rate is conducting using a solvent shift/quench approach (Vandecruys et al., 2007; Warren et al., 2010; Yamashita et al., 2011). A supersaturated system is generated by adding the drug dissolved in a water-miscible organic solvent to an aqueous solution of the excipient of interest present at a concentration of 2.5% (w/v) at an appropriate pH. The concentration achieved in this system as well as the rate of precipitation is assessed analytically using either nephelometry or filtration followed by UPLC (Ultra performance liquid chromatography) as a function of time. Excipients usually used in this assay include rheological and other polymers including cellulosic systems, surfactants, cyclodextrins and related materials. Both the extent and stability of the formed supersaturated systems is important in assessing the possible use of the excipients as components in the amorphous solid dispersion-based formulation. Once this excipient space has been assessed, these materials are used to form films with the API. This exercise is intended to interrogate API-excipient miscibility in the solid state as well as the tendency for the dispersed API to remain as such over time (that is to maintain the dispersion without phase separation or recrystallization) (Janssens et al., 2010; Weuts et al., 2011). Films are case out of a common solvent (a solvent providing for good solubility of both the API and polymer/excipient) and the formed film is analytically assessed (usually using white light and birefringence microscopy, XRD and DSC). In addition, the dissolution properties of the films are assessed to see whether supersaturation occurs and whether the API remains in solution. These tasks are performed in an automated manner using specially designed 96-well plates to allow for the analytical assessments as well as the dissolution evaluation (Brewster et al., 2011). The microscopy can suggest changes in the degree of miscibility including phase separation and crystallization while XRD and DSC are confirmatory method to assess any increase in crystalline content over time.

The application of these approaches to a poorly water-soluble drug candidate can be illustrative. A drug candidate has a melting point of 270 °C and a measured T_g of 113 °C with a molecular weight of 433 g/mol. The compound has a pK_a of 3.5 and a solubility in simulated intestinal fluid of 0.6 μg/mL. Excipient screening studies were completed using the automated 96-well plate method. In this screen a total of 41 excipients were assessed alone or in combination at four different concentrations. The study suggested that the following materials provided for significant effects on the extent and duration of supersaturation: Solutol, Poloxamer 407 and TPGS. Films then were cast using the automated protocol in

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which 95 excipients or excipient combinations were assessed based on unitary, binary or ternary systems at two API to excipient(s) ratios. Hits from the precipitation inhibition screen were included in the film experiments. Identified dispersions which were amorphous and stable over time included: API:HPβCD:TPGS, API:HPβCD:HPMC, API:HPMC:Poloxamer 407, API:HPMC-P:Poloxamer 407 and API:HPβCD:Solutol. Dissolution profiles were completed for films that performed well in stability and physicochemical property assessment using a 96-well two-stage dissolution approach in which simulated gastric fluid is added at time 0 followed by FaSSIF, which was added at 60 min. This protocol assessed supersaturation under conditions which might represent the stomach to intestine transition. Dissolution profile for the API as well as for cast films suggested an increasing supersaturation tendency in the order API < API:HPMC < API:HPMC;TPGS < API:HPβCD:HPMC. In aggregate, the data suggested that the most robust system was the API:HPβCD:HPMC in terms of (1) manifesting good supersaturation, (2) showing minimal precipitation upon SGF-FaSSIF transfer and (3) demonstrating insensitivity to composition. These systems were then used to configure test formulations to assess biopharmaceutics in the rat. Formulations involved spray drying the ingredients and administering the spray dried powders to rats by gavage in a 0.5% methocel suspension. The test formulations administered in this way included: API:HPβCD:TPGS (1:3:1), API:HPMC:TPGS (1:3:1), API:HPMC (1:1), drug milled into the nano-domain and micronized drug. Using the micronized suspension as a reference, all of the enabled systems provided for varying degrees of benefit. The nano-sized suspension was almost 3-fold more bioavailable than the micronized suspension while the HPMC dispersion gave almost 4-fold higher exposure. Consistent with the dissolution profiles, the best formulation in the rat was the API:HPβCD:TPGS which gave an oral bioavailability almost 9-fold greater than that of the simple micronized drug. These rough designs were then converted to formulations that might be tested clinically. These more refined systems included: (A) a spray-dried solid dispersion of API:HPβCD:TPGS filled into a gelatin capsule, (B) a dispersion of API:HPβCD:HPMC coated onto an inert Mono-N-carboxymethyl chitosan sphere using a closed Wurster process and filled in a capsule, (C) a solid dispersion of API:HPMC pressed into a tablet, (D) a nanosuspension-based tablet and (E) API in capsule. These systems were then assessed in a traditional USP II dissolution apparatus using a two-phase transfer model approach. Not only did the optimized formulations retain the dissolution profiles of the simple dispersions from where they were derived, but the USP II data were well correlated with the 96-well two-phase dissolution method. Importantly, the oral bioavailability assessment seen in rats of the simple systems was maintained in other animal models with the optimized dosage forms.

12.7. Liquid-filled capsules (lipids/surfactants/S(M/N)EDDS/solvent) feasibility

A third possible enabling formulation trajectory included solvent-, lipid-, surfactant- or lipid/surfactant-based systems. These are important in the industry with an estimated 2–4% of marketed oral dosage forms using these concepts. Key questions that need to be answered regarding the possible use of these technologies include whether the intended dose can be solubilized in an appropriate volume of vehicle (capsules usually limit this to 1 mL) and at an appropriate pill burden and whether the API so solubilized is physically and chemically stable over time. In addition, if a S(M/N)EDDS (Self microemulsifying/nanoemulsifying drug delivery system) is intended, does the system perform *in vivo* as designed. The pharmaceutical questions are assessed using an automated, down-scaled workflow based on viscous liquid handling. Solvent systems showing good solubility can then be added to water to assess emulsifying or self-emulsifying properties based on a two- or three-phase diagram. Isotopically clear systems indicative of micro/nano-emulsification can be assessed using white light and birefringence microscopy.

A case study optimizing a poorly water-soluble drug candidate as a self-emulsifying system was addressed. The compound gave poor exposure when dosed as a suspension compared to a solution (a relative bioavailability of 1.7%). Precipitation inhibition screening using a 96-well plate assay suggested that the best materials were surfactants with the three most important hits being TPGS, Cremophor RH40 and Tween 20. These materials were then included in a larger SEDDS (self-emulsifying drug delivery system) screen which made used of a list of 50 lipids and surfactants in various ratios. In this assay, solubility was optimized with 18 hits giving solubilities >50 mg/g. These hits were then screened for biopharmaceutical properties in the rat and compared with a standard solution (drug dissolved in TPGS/NMP). Based on the drug solution, 5 formulations gave significant increases in exposure with the best formulation containing Capmul PG8, Cremophor RH40 and lauric acid. This formulation gave a relative bioavailability of 165% versus the drug solution and increased the absolute bioavailability to almost 50%.

12.8. Predictability of animal models for humans

While these factors target API and pharmaceutical aspects in formulation finding, an assessment of biopharmaceutical properties are likewise of interest. The selection of an appropriate animal model to assess these formulation concepts is complex (see sections above) and is impacted by an amalgam of API, pharmaceutical and biopharmaceutical features. Wu et al. (2004) suggested that the dog was a useful model for assessing nanosuspension based on both translate-ability to man as well as the ability of the dog model to predict food effects attenuated by the nanosuspension approach (Wu et al., 2004). The model compounds assessed in these studies were MK-0869 (aprepitant). By contrast, Mackie et al. (2009) and Ouwerkerk-Mahadevan et al. (2011) suggested that the rat was a better model to assess nanosuspension based on comparison of rat, dog and human clinical data (Mackie et al., 2009; Ouwerkerk-Mahadevan et al., 2011). In these assessments, two compounds were evaluated and the alignment of rat and human data were thought to be related to pH difference in the rat and dog GI tract as well as the more human-like transit time. The differences in outcome of these two sets of studies may be related to the API chemotype assessed in that aprepitant (pK_a = 9.7, MP = 254 °C, log$P$ = 4.8 and water solubility of 3–7 μg/mL) and the compounds from the second set of studies (Compound A, pK_a = 3.45, MP = 216 °C, log$P$ = 3.1, water solubility <0.1 μg/mL; Compound B, pK_a < 2, MP = 156 °C, log$P$ = 4.6, water solubility <0.1 μg/mL) differ in several important respects. These include the pK_a and water solubility potentially biasing either the rat or dog to be the better model of translation.

Newman et al. (2012) discussed the application of various *in vivo* models in the assessment of solid dispersions (Newman et al., 2012). In their retrospective analysis of 40 studies, the following animal models were described including the dog (41%), rat (24%), rabbit (15%) and monkey (2%). In some cases, the dog model was altered using agent to modify the GI pH. *In vitro–in vivo* relationships could be constructed using animal and dissolution data in all but 1 rat study, 3 rabbit studies and 2 dog studies. Generally, selection criteria of the animal model for a particular formulation is most often aligned with biopharmaceutics (including physiological and metabolic similarities to man, translate-ability to man) and less on pharmaceutical and API properties. Nonetheless, the appropriate selection of an animal model will

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require all three axes to be incorporated into a decision tree including comparisons of fluid volumes as a function of GI location (as well as when comparing the fed and fasted model) as well as GI regional pH, composition and ability to support supersaturation. In a line of reasoning similar to the nanosuspension discussion the selection of an animal model for amorphous solid dispersion is best completed on a case-by-case basis – factors to be taken into account include also the relevant API and its pharmaceutic and biopharmaceutic properties. Other species such as the pig and minipig may also be of value in these assessments in addition to the more generally applied models (see Section 4).

Lipid-based formulations have a number of factors in common with the other formulation types described but also a number of significant differences. In common with other approaches, formulation processing can generate supersaturation which may be an important system aspect for enablement (Brouwers et al., 2009; Williams et al., 2013). By contrast, lipid formulations may also be processed by digestion and the API-lipid or digestion products may be useful modalities for targeting lymphatic absorption. The importance of these factors means that more animal model development and optimization have to be completed as a function of the pharmaceutical axis for these systems. In addition since a number of chemotype rules have been postulated related to log*P*, molecular weight and lipid solubility requirements, more API insight is also applied to the selection of an animal model. Generally, rat and dog studies are used for all three assessments. The dog is more commonly used to assess the effect of lipid on absorption consistent with its use in evaluating food effects however models for lymph duct cannulation are available for both species (Porter and Charman, 2007). Dogs also have the advantage that human-scaled dosage forms can be administered (Charman et al., 1997; O'Driscoll, 2002).

As the use of enabling formulations increases and as API's becoming increasingly difficult to formulate, appropriate animal models to study these systems are essential. While historically, the focus associated with selecting an animal model has been biopharmaceutical in nature, more and more, factors associated with model alignment as a function of API properties and formulation processing will likely grow in importance. This is based on both the need for increased translation from the preclinical species to man but also in an effort to generally reduce the number of animals used in formulation optimization and drug development. *In vivo* factors associated with supersaturation, excipient processing and API uptake at the intestinal mucosa will also have increasing impact on the choice of a useful animal model (Bevernage et al., 2012; Brouwers et al., 2009; Takano et al., 2010).

In contrast to the various animal models being available to make predictions of *in vivo* dissolution and permeability for IR formulations in humans, to date, there is no established animal model published that would reliably predict colonic API stability (microbiota), permeability, and dissolution. A biopharmaceutical colon model would be of high interest for the developability assessment of slow release formulations. Human microflora consisting of 10–100 trillion cells, from ~160 species is a complex mixture which requires certain technical know-how to culture. Also, it is important to keep the *in vitro* culture media to the most physiological relevant composition to allow the microbiota to exhibit their natural enzymatic activity (Qin et al., 2010). Experimentally, *in vitro* API stability and dissolution experiments have been performed (www.TNO.nl.pharma; www.prodigest.eu), however, the number of compounds of which colonic stability and dissolution data have been validated against human PK data are very limited, and respective *in vivo* animal and human colonic absorption and/or dissolution data are not publically available to an extent that would allow the generation of a predictive biopharmaceutical colon model.

13. Overall gap analysis

This review has made clear that there are important gaps in our understanding of the human GIT as well as the physiology of animals that are currently used for *in vivo* drug and formulation characterization.

Typical gaps in knowledge about human GI physiology affecting dosage form performance have been mentioned in this review including e.g. intraluminal water availability in particular in segments that are low in water content but important for dosage form performance and absorption such as the small and large intestines. Likewise little information is available on the magnitude and frequency of intraluminal pressures and hydrodynamics that act as mechanical stress factors and thus affect the integrity of dosage forms (disintegration, erosion). This information is however needed in order to design more meaningful *in vitro* test systems that are reflective of the *in vivo* situation. Relevant for the active ingredient itself is the question about its mechanism of intestinal permeation and possibly metabolism. In recent years some progress has been made but not sufficient for a clear understanding about carrier-mediated and passive components of drug absorption in the intestine. Identification of carriers, their expression along the GIT and relationships between expression and drug affinity are just some examples where progress needs to be made. This holds true for humans but also for most of the animal models that are used for drug and dosage form characterization. Important differences e.g. in gastric and intestinal pH, gastric emptying and intestinal transit times and motility as well as intestinal permeability may be taken as the underlying cause for differences between animal species and insufficient predictability of the effects in humans. An analysis of databases covering different formulations and drugs in different species is needed in order to better define the relationships between chemical space of the compound, formulation space and usefulness as screening tool and predictability for humans. Imaging tools for animals and for humans represent important tools to investigate some of these phenomena and need to be established further.

Progress needs also to be made in particular for understanding and better predicting the activity of compounds exerting low water solubility in order to design formulations that will predictively deliver their active ingredients in various patient groups. Among these factors, variability in composition and relevance of GI fluids for drug solubilization should be mentioned. On the same token little is known about the intraluminal behavior of formulations, i.e. the concentrations of drugs following oral administration of their formulations in the lumen of the stomach and the small intestine, their solubilization and precipitation also as a function of chemical structure (acids versus bases versus neutral compounds) and sites (stomach versus small intestine) and dose. This knowledge is needed to understand the performance of enabling formulations for low soluble compounds as well as to better reflect the solubility/permeability relationships that result from the dissolution and solubilization of the API and its permeation across the intestinal epithelium. Ignorance of these items leads to a trial and error approach in the design of *in vitro* experimental conditions that may or may not reflect the *in vivo* situation appropriately.

In particular for controlled release dosage forms the knowledge about their *in vivo* transit and processing as well as the absorption of the released drug in different intestinal segments is crucial but frequently not available. Thus sufficient resources may be spent in vain trying to develop formulations for compounds that intrinsically are not prone to be delivered in oral sustained release systems simply as a consequence of their cumbersome absorption and metabolism properties.

Another wide open field is the personalized medicine approach and the need to predict drug product performance not just in

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healthy volunteers but also in the target patient group. For that purpose, our knowledge gaps with respect to factors that differ in patients versus volunteers and that are important determinants for drug bioavailability need to be closed.

Needless to say is that our current knowledge on the predictability of *in vivo* effects of excipients used for formulating oral drug products as well as interactions between active pharmaceutical ingredients and these excipients or food components or neutraceuticals is still underdeveloped. Progress in these areas will have important consequences not just for the development of optimized drug products but also for streamlining the regulatory decision making process since it can be done based on sound scientific facts rather than being the result of sometimes overcautious regulatory expectations putting the safety as the only and overall decision guiding principle. For that purpose the concept of *in vitro–in vivo* correlations needs to be expanded in various directions, for example by making best use of the relationships found between *in vitro* dissolution and *in vivo* pharmacokinetics.

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Conflict of interest

The authors disclose no conflicts. M.K. is also employed at the Medicines Evaluation Board of the Netherlands, but the views presented here do not necessarily reflect the opinion of the Board. A.L. is employed at the Medical Products Agency, Uppsala, Sweden, but the views presented here do not necessarily reflect the opinion of the Agency.

14. Uncited reference

Q5 Lennernäs et al. (1993).

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