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Integration of advanced methods and models to study drug absorption and related processes: An UNGAP perspective

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SUMMARY

This collection of contributions from the European Network on Understanding Gastrointestinal Absorption-related Processes (UNGAP) community assembly aims to provide information on some of the current and newer methods employed to study the behaviour of medicines. It is the product of interactions in the immediate pre-Covid period when UNGAP members were able to meet and set up workshops and to discuss progress across the disciplines. UNGAP activities are divided into work packages that cover special treatment populations, absorption processes in different regions of the gut, the development of advanced formulations and the integration of food and pharmaceutical scientists in the food-drug interface. This involves both new and established technical approaches in which we have attempted to define best practice and highlight areas where further research is needed. Over the last months we have been able to reflect on some of the key innovative approaches which we were tasked with mapping, including theoretical, *in silico, in vitro, in vivo* and *ex vivo*, preclinical and clinical approaches. This is the product of some of us in a snapshot of where UNGAP has travelled and what aspects of innovative technologies are important. It is not a comprehensive review of all methods used in research to study drug dissolution and absorption, but provides an ample panorama of current and advanced methods generally and potentially useful in this area.

This collection starts from a consideration of advances in *a priori* approaches: an understanding of the molecular properties of the compound to predict biological characteristics relevant to absorption. The next four sections discuss a major activity in the UNGAP initiative, the pursuit of more representative conditions to study lumenal dissolution of drug formulations developed independently by academic teams. They are important because they illustrate examples of *in vitro* simulation systems that have begun to provide a useful understanding of formulation behaviour in the upper GI tract for industry. The Leuven team highlights the importance of the physiology of the digestive tract, as they describe the relevance of gastric and intestinal fluids on the behaviour of drugs along the tract. This provides the introduction to microdosing as an early tool to study drug disposition.

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Microdosing in oncology is starting to use gamma-emitting tracers, which provides a link through SPECT to the next section on nuclear medicine. The last two papers link the modelling approaches used by the pharmaceutical industry, in silico to Pop-PK linking to Darwich and Aarons, who provide discussion on pharmacometric modelling, completing the loop of molecule to man.

Abbreviations

ADME	absorption, distribution, metabolism and elimination
API	active pharmaceutical ingredient
BCRP	breast cancer resistance protein
CT	computerised tomography
CYP	cytochrome P450
DP	drug product
EOM	equation of motion
FaSSIF	fasted state simulated intestinal fluid
FDA	Food and Drug Administration
GI	gastrointestinal
IVIVC	in vitro-in vivo correlation
MD	molecular dynamics
PBBM	physiologically-based biopharmaceutics
PD	pharmacodynamics
PET	positron emission tomography
PK	pharmacokinetics
pop-PK/PD	population pharmacokinetics/pharmacodynamics
PSA	Parameter Sensitivity Analysis
RT	regular tablet
SPECT	single photon emission computerised tomography
UNGAP	Understanding Gastrointestinal Absorption-related Processes

1. Introduction

Since the first development of a treatment for disease, medicines have been administered via the gastrointestinal tract, as the outcome of surgery was often fatal. Texts from the Babylonian-Assyrian culture (2000 BCE) describe a pulverised mix of seeds, juices, plant resins and leaves extracted into beer or wine. Medicine was associated with magic, with the belief of deities intervening in the probable success (Castiglioni, 2019). Medicine was assessed by outcome rather than rational explanation and measurements were shaped by observations of toxicity. Posology developed through accidents, or rather the wish to avoid death and the appearance of the unit dose form appeared in Egypt, with pills made from medicinal herbs mixed with honey, bread flour and animal fat and rolled from dough with the fingers as described in several papyruses.

Better analysis of the behaviour of medicine started with the ability to measure safely drug concentrations in biological fluids and the emergence of pharmacokinetics. The hospital became the laboratory to compare drug concentration-time profiles from parenteral and oral doses and calculate treatment in renal and hepatic impairment. Ultimately, many hospital-based technologies such as x-rays, fluoroscopy, gamma scintigraphy, ultrasonography and magnetic resonance imaging were borrowed by pharmaceutical investigators as the tools to investigate the behaviour of medicines and the link between imaging and investigation of dosage forms *in vivo* became firmly established.

The particular interest has always been oral administration. Although parenteral administration usually results in higher plasma concentration, oral administration of drugs has been the preferred way of delivery, mainly because of convenience. This route does not require specialised personnel for the application, or robust patient training as may be needed for nasal, inhaled or transdermal products. Incorrect technique can result in large variations of drug levels and the oral route is preferred even if in some cases a rectal application might result in control that is more effective.

Generally, the development of the drug and the formulation suitable for oral administration has been the main challenges during pharmaceutical drug development. This is one of the most time consuming and probably the major obstacle in the medicine's development process. An important number of drug candidates reach the market only after many years of pre-clinical and clinical testing of different formulations. Many of course fail before reaching Phase III. Uncertainty exists regarding the time when the maximum plasma level is reached, as this parameter varies for different drugs over a wider range after oral administration. The development of an *in vitro-in vivo* correlation (IVIVC) becomes important and to optimise an oral preparation requires a deep knowledge of the amounts and properties of the fluids of the gastrointestinal tract and the properties of regional absorption. This only can be achieved by using adequate methodologies to simulate, predict and measure, not only the stability and transit of the drugs and excipients whilst within the gastrointestinal tract, but also to assay and determine the efficiency of absorption, using a knowledge of gut motility to mimic the right gut conditions to optimise these processes.

2. Molecular dynamics description of the process of gastrointestinal drug absorption

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- Molecular dynamics (MD) modelling is a useful tool to complement and predict experimental findings of drug absorption at microscopic level.
- The section describes the principles of MD in simulating the influence of chemical composition and physical properties of the components of a formulation, including pH, milieu properties, and interactions with biological proteins that influence mucosa drug penetration.
- A description of several examples of MD simulations, where factors as pH, bile salts, and interactions with GI fluid and mucosa are considered, gives the reader a deeper understanding of the power of these computational protocols.
- The section concludes on the synergy of MD methods with other experimental techniques to understand drug absorption.

Summary

Molecular dynamics (MD) is a simulation technique, which can be employed to reveal various microscopic aspects of GI drug absorption. The results from MD modelling may be used to complement experimental findings and thus ensure a more comprehensive representation of the process. The current section of the review introduces the essentials of the method and summarizes the outcome from molecular dynamics simulations addressing various aspects of drug behaviour within the GI system. Because of these studies, we have obtained knowledge about the chemical composition and shape of drug nanocarriers and how these are influenced by pH. Furthermore, the mechanism of aggregation of bile salts and of solubilisation of drugs therein has been elucidated. The most important interactions between drugs and protein receptors can be identified, and geometric and chemical parameters influencing the penetration of drugs across intestinal mucosa can be outlined. There are many other open questions, which could still be addressed by molecular dynamics. With the rapid development of computational power, this approach could become a standard part of the methodological toolbox used to decipher how drugs are absorbed *in vivo*.

2.1. Introduction

A comprehensive advanced technique, which may be used to elucidate microscopic details related to drug absorption in the GI tract, is referred to as MD. MD is a statistical mechanics method that relies on solving the Newtonian equations of motion (EOMs) to propagate a (multimolecular) model system in time (Allen and Tildesley, 2017). The EOMs are solved numerically for each particle and thermostats and barostats are used to maintain constant temperature and pressure during the simulations. In classical molecular dynamics, energy and forces needed for the EOMs are obtained from molecular mechanics force fields (FFs). The model systems may be treated at various degree of detail. In fully atomistic models, each atom represents a separate particle exerting and subject to a force in the EOMs. In the so-called coarse-grained (CG) approach, several atoms are united into a bead and beads interact with each other. The important aqueous environment may be treated either implicitly (representing it as a dielectric continuum) or explicitly (by inclusion of solvent molecules in the simulations). The latter yields much more accurate results because all specific intermolecular interactions are taken into account but it is also very computationally costly. Molecular dynamics can provide structural, mechanistic, thermodynamic, and temporal insight into the processes taking place in the investigated system.

Bearing this in mind, the applicability of MD simulations to reveal the specifics of GI drug absorption are reviewed next, following a set of factors.

2.2. Molecular dynamics and drug absorption

The absorption of drugs into the GI tract of the human organism could be controlled better if understood at the molecular level. There are several key factors that govern the process at this level (Golub et al., 1986; Augustijns et al., 2020). First are the physical-chemical properties of the single drug molecules, which are relevant for the process of GI absorption – examples are atomic charges, energy and type of molecular orbitals, dipole moment, polarizability, number of atoms able to form hydrogen bonds. Other aspects are the specifics of the drug formulation; its stability, the effect of pH of the medium, the dissolution capacity of the carrier material, and the efficiency and time of release of the drug. Relevant issues are also the interactions of the drug with various components of the GI fluid, mostly bile salts, digestion enzymes, and other macromolecules on the mucosa as well as the capacity of the drug to permeate the intestinal mucosa, either by passive or active diffusion, entering the blood stream. Finally, the available surface area of the bowel, the transit time of the drug in the intestine, and the flow characteristics, such as rate, viscosity, etc., of the GI fluid are important determinants that should be also considered in any modelling. These aspects are discussed below within the context of the relevant simulation techniques.

2.2.1. The physical-chemical properties of the single drug molecules

The single-molecule characteristics are usually estimated with simple and fast computational methods for libraries containing a large number of drug candidates. Then, they are related to experimentally measured bioactivities by statistical Quantitative Structure-Activity Relationship (QSAR) models to determine the essential molecular characteristics responsible for the experimentally measured drug efficiency. Such evaluations have been performed on a huge variety of drugs (a recent interesting review is provided by Bergström et al., 2016) and there are also some examples (Lee et al., 2020; Oja and Maran, 2018; Levitt, 2013; Geerts and Vander Heyden, 2011; Klopman et al., 2002) focused on the capacity for GI drug absorption. The studies related to the physical-chemical properties are carried out most often on single drug molecules in the gas phase, hence, do not include explicitly environmental effects and do not provide dynamic picture of the drug absorption process.

2.2.2. Specifics of drug formulations and effect of the GI medium thereon

It has been shown theoretically how various pharmaceutical formulations behave in conditions resembling those in the GI system or how drug molecules are released from them or structured within a drug delivery system (DDS) (Boyd et al., al.,2019; Exner and Ivanova, 2020). Some of the studies highlight the importance of a drug carrier composition (Le et al., 2018) or shape (Yu et al., 2018) for proper delivery of drugs to or within the GI system. Le and colleagues discovered using MD simulations {GROMOS 51a8 FF, NVT ensemble with $T = 310$ K, 200 ns trajectories} that nanoparticles with preassembled paclitaxel drug core enveloped with tannic acid as intermediary and N-vinylpyrrolidone as the outermost shell could be successful in stabilizing the drug until it reaches the GI system (Le et al., 2018). Yu and colleagues employed CG MD {Lennard-Jones potential for all beads, bound terms for nanoparticles, NVT ensemble with $T = 0.23\varepsilon$, $2 \times 10^6\tau$ long trajectories, 4 independent runs} to demonstrate that semi-elastic poly(lactic-co-glycolic acid) nanoparticles (each containing 108 oligomer chains) are better drug GI carriers than hard or soft ones because of more enhanced penetration inside the mucus layer (modelled as cross-linked polymer chains on a 2D regular mesh). Bao and colleagues also treat mucus penetration by peptosomes and release of curcumin thereof by CG MD (Bao et al., 2020). They outline the short nanotube shape as the most appropriate. These studies are carefully carried out and long enough. They provide guidelines about the importance of the polymer corona of the nanocarriers and that more elongated shapes should be sought. The research could be extended further into obtaining also atomistic details of the intermolecular interactions within the formulations.

The effect of pH and of the interaction of drug-loaded formulations with components of the GI medium is highlighted in several studies (Katiyar and Jha, 2018; Suys et al., 2017; Birru et al., 2017). Katiyar and colleagues investigated by MD {CHARMM27 FF, NVT ensemble with $T = 298$ K, 30 ns trajectories} the aggregation of 74 molecules of poly (acrylic acid) 20-mers upon pH increase, reflected by different degree of deprotonation of carboxylic groups, in the absence and in the presence of various GI ingredients: NaCl, NaOH, CH_3COONa, CH_3COOH, maleic acid, or sodium oleate. The influence of loaded doxorubicin on the structure of the carriers was also tested in this study. The obtained results suggested diminished aggregation at higher pH. This was interpreted as better drug release in the GI, compared to gastric environment where the pH is lower, larger aggregates would form and retain the drug longer. Sustained release of doxorubicin in the GI system is predicted.

Models of the GI fluid itself are also set up (Suys et al., 2017; Birru et al., 2017) and the effect of cholesterol or glyceryl-1-monooleate (GMO) or the presence of the poorly water-soluble drug danazol on the intermolecular self-assembled aggregates is checked by MD {GROMOS 53a6 FF, SPC water, NPT ensemble with $P = 1$ bar and $T = 310$ K, 100 ns or 200 ns trajectories}. Variations of pH in the range 1–6 are also applied (Suys et al., 2017). The GI medium is represented by the glycodeoxycholate (GDX) bile salt together with a 4:1 mixture of digested lipids (LPC) and oleic acid (OA). While continuous phases are stable below pH 3, separate micelles of decreasing size emerge at higher values. There, predominantly mixed GDX/LPC/OA oblate micelles are formed with GDX and LPC mostly located on the surface. Cholesterol simply includes into these micelles and has no measurable influence on their size or shape. In contrast, addition of GMO markedly changes these parameters. Upon increase of its concentration, the mixed micelles become larger and more rod-like until transition to continuous hexagonal phases takes place. The presence of GMO is suggested to facilitate solubilization of the drug. Danazol was preferentially buried into the hydrophobic core of the mixed micelles. The drug resided on the surface of a micelle only if forced by small micelle size. It was concluded that the drug does not influence the aggregates structure, similar to cholesterol. The same digested lipid/fatty acid model of GI milieu was used to determine by molecular dynamics the partition coefficients of 22 different popular drugs between a mixed micelle and the aqueous solution (Turner et al., 2012). An advantage of these studies is the use of

atomistic and united-atom force fields. At the same time, however, this limits the length of the trajectories. The modelled systems are also quite limited in number and very specific. Hence, in order to obtain generally valid conclusions about the distribution of the separate components into drug formulations or about the effect of pH, a much larger database will have to be accumulated.

The aspect of solubility of drug formulations or of free drugs in the GI fluid may also be addressed, albeit indirectly, by molecular dynamics simulations. A recent review provides a nice overview of the available MD-based methods (Hossain et al., 2019). They are all essentially based on estimating solubility of the drug material by calculating the free energy of transfer between two media. Some examples were summarized by Bergström and Larsson (Bergström and Larsson, 2018). Róg and Bunker, 2020, highlight a number of compounds for which solubility in various solvents is determined with the help of molecular dynamics simulations.

2.2.3. Interaction with components of GI fluid and mucosa

2.2.3.1. Bile salts. Since bile salts (BSs) are a major ingredient of the GI system fluids, their self-assembly or interactions with drugs or receptors are also studied by MD simulations. Harmat and colleagues (Harmat et al., 2019) generated molecular dynamics trajectories {OPLS-AA FF, TIP3P water, MUMO approach with temperature averaging or 500 ps of targeted MD} based on experimental NMR restraints to determine ensembles of stable conformations of the bile-salts-binding gastrotropin receptor in *apo* state or with bound glycodeoxycholate or glycochenodeoxycholate. Using the obtained structural data, the mechanism of binding of bile salts is elucidated. It is predicted that the BS binding process involves two modes of barrel opening of the protein. The cooperativity of action of the bile salts upon binding was verified.

An MD study {AMBER03 FF, TIP3P water, 120 mM NaCl, NVT ensemble with $T = 310$ K, 150 ns trajectories} by Mustan and colleagues (Mustan et al., 2015) focuses on the mechanism of aggregation of six BSs – cholate, deoxycholate, and their glyco- and tauro-derivatives. Intermolecular hydrophobic interactions are outlined as the main stabilizing factor for the obtained ellipsoid aggregates, characterized with dynamic intermolecular arrangement and very hydrophobic core. The aggregation time and the size and shape of the micelles depend critically both on the number of hydroxyl groups in the steroid skeleton and on the side chain substituent. This chemical specificity of BSs is confirmed in the work of Li et al., 2016. There, the interaction of cholate, deoxycholate, chenodeoxycholate, and ursodeoxycholate with the drug nevirapine is modelled with MD {CHARMM FF, SPC/E water, NVT ensemble with $T = 310$ K, 100 ns trajectories}. Models of 1:1 or 2:2 drug:BS ratio clusters in explicit water are simulated. Much tighter association of the drug with deoxycholate than with cholate is observed and attributed to better spatial fitting of the molecules, leading to intermolecular stabilization by hydrogen bonds. The results, however, could not be linked to the contrasting experimentally observed capacity of the BSs to prevent crystallization of the drug in the GI tract. Prakash and colleagues reveal by MD simulations {CGenFF FF, no water, NPT ensemble with $T = 310$ K and $P = 1$ atm, 60 ns trajectories} the interaction of ibuprofen in three different milimolar concentrations with cholate or with a cholate-decorated lipid micelle formed from 60 molecules of single-tail dodecylphosphatidylcholine (Prakash et al., 2012). It is shown that the drug and cholate always aggregate into mixed micelles. Ibuprofen induces the formation of much larger (up to 20 molecules) mixed micelles than known for pure cholate (ca. 5 molecules) but the shape remains elongated. During this analysis, it was observed that smaller pure drug and cholate clusters were formed first, which then fused to make the mixed micelles. It was concluded that the drug also adsorbs on the surface of the preformed cholate-decorated lipid micelle but does not mix with cholate in that location. The computational findings were experimentally verified. The smaller ibuprofen aggregates on the lipid micelle surface are put out as a reason for its reduced toxicity within lipid-assisted formulations. This group of fully atomistic studies provides a rather consistent description of the mechanism of aggregation of bile salts. Guidelines for the solubilization of several drugs into bile salts micelles are also obtained.

2.2.3.2. Endothelial receptors. MD simulations are used to characterize the molecular structure of drugs acting in the GI system or of preformed drug-receptor complexes. Brancale and colleagues obtained by MD {GROMOS FF, TIP3P water, NPT ensemble with $T = 310$ K and $P = 1$ atm, 3 independent trajectories of 400 ns each} representative conformations at various pH values of peptide drugs (plecanatide and linaclotide), which bind the guanylate cyclase-C receptor regulating the intestinal activity (Brancale et al., 2017). The geometries of the two peptides are compared to that of enterotoxin, which has very high affinity for the receptor. Based on this comparison, it is predicted that linaclotide will be very active toward the receptor at all pH values while plecanatide will be able to bind the protein only at pH 5. Two amino acids are identified as key for stabilizing the bioactive conformation of plecanatide at this pH. The diminished activity of the drug is attributed to more pronounced pH-dependant structural flexibility. The obtained computational results are nicely linked to the known therapeutic behaviour of the two drugs. Meduru and colleagues combine docking, pharmacophore identification, and MD simulations to predict active inhibitors of the receptor dipeptidyl peptidase-4 present in the GI tract, which could aid diabetes treatment (Meduru et al., 2016). A series of 82 small molecules was screened to filter out those with the highest affinity for the receptor. The docked drug-receptor complexes of the 15 best candidates and of 3 approved drugs used as reference are simulated with MD {CHARMM FF, explicit water, NVE ensemble, 1 ns trajectories}. It is shown that all ligands are stably bound in the protein pocket, stabilized by up to 12 intermolecular hydrogen bonds, and the key interacting amino acids were identified.

2.2.4. The capacity of the drug to permeate the intestinal mucosa

The process of drug penetration through the intestinal membrane has been another object of MD simulations since it has microsecond characteristic times (Ivanova et al., 2015). It is also very important for the GI drug absorption. However, there are many aspects at the molecular level, which are still not elucidated. Some of the most important questions are: influence of the membrane composition or of the ionization state of the drug (governed by pH) on the penetration; effect of other environmental factors, e.g., ionic strength or concentration gradient; molecular mechanism and energetics of the translocation process. Surprisingly, there are only a few MD studies reporting results related to drug permeation of intestinal membranes. They are aimed at elucidating the mechanism of penetration of drugs or DDSs.

An MD study {OPLS-AA FF, TIP3P water, 150 mM NaCl, NPT ensemble with $P = 1$ bar and $T = 310$ K, no trajectory length specified} described the interaction of the poorly-water-soluble drug piroxicam, two neutral tautomers, one zwitterionic and one negatively charged form, with standard and PEGylated lipid model membranes (POPC, POPC-cholesterol, or POPC/DSPE-PEG) to understand better the bioavailability of the pharmaceutical and the capacity to include it into liposomes for delivery (Wilkosz et al., 2017). It is demonstrated that the neutral forms of the drug hardly penetrate the pure POPC layer, while the charged ones diffuse in and out. The presence of cholesterol reverses the situation. In the PEGylated layers, smaller number of drug molecules (neutral or charged) is translocated overall. The reason is enhanced restraining of piroxicam in the PEG corona. The preferred location of the drug is in the region between lipid heads and tails, the particular positioning modulated slightly by charge. Significant rotational variability is observed, limited somewhat by the presence of cholesterol. Cluster formation of the drug molecules in solution takes place, which delays their membrane translocation.

Thanki and colleagues monitored the membrane permeability of the antibiotic amphotericin B in free state and conjugated to oleic acid, the latter being aimed at reducing its toxicity and improving the membrane translocation capacity (Thanki et al., 2018). MD simulations {Lipid11/Gaff FFs, explicit water, NPT ensemble with $P = 1$ atm and $T = 310$ K, 50 ns (embedded) or 100 ns (free) trajectories} of four (two head-to-head and two head-to tail) prebuilt intermolecular dimers of the conjugate are performed first. Then, the two stable ones (both of them head-to-head) are embedded into a model POPC:sterol symmetric bilayer. The same is done with drug:sterol complexes. Ergosterol or cholesterol are tested as the steroid counterparts of complexes and membranes. It is established that the complexes with ergosterol of the free drug and of the conjugate are more stable inside the membrane than those with cholesterol. Unlike the monomers, the intermolecular dimers of the drug show lack of selectivity, interacting comparably with the two sterols. Only one of the dimers of the conjugate is selective for ergosterol. The computational data match the outcome from experiments conducted in the same study.

The penetration studies appended in a very suitable way the overall microscopic description of drug absorption in the GI tract.

2.3. Conclusions

As evident from the above synopsis, the aspects related to drug interaction with components of the GI fluid and to mucosal permeation capacities of GI drug absorption are tackled only in a few instances with MD simulations. Given the greater potential of molecular dynamics to model binding or interaction of drugs with biomolecules (of the order of 10^4 publications) or of drug penetration across lipid bilayers (more than 2000 publications) in principle, much more applications with focus on the GI part of drug action are to be expected.

Other factors, like the estimation of the surface area of the bowel, transit time of the drug in the intestines, and flow characteristics of the GI fluid have much longer characteristic times, and can therefore be treated only with coarse-grained or even mesoscale MD simulations. The several existing studies (Alberini et al., 2018; Marbach et al., 2018; Zeuthen et al., 2016; Tchesnokova et al., 2010; Haddish-Berhane et al., 2006; Yu et al., 2018) report only specifics of transport across channels upon shear-induced intestinal epithelium adsorption, drug release from a DDS in the colon, or drug transport along a model GI tract.

The limitations of molecular dynamics methodologies stem mostly from its classical nature, from the availability of the force fields, and from the computational resources demanded. The classical approximations within the MD method prevent it from studying systems and processes of quantum origin, e.g., ground-state properties (at low temperatures), charge transfer via tunnelling, supercritical phenomena, or chemical reactions. This could be overcome with the so-called QM/MM simulations. There, the key (but smaller in size) part of the model system is treated with a quantum chemical method and the rest is described with a force field. Contemporary force fields enable accurate description of most bioavailable macromolecules but parameters are still in demand for many drug candidates. The simple potential forms may also influence the accuracy of the obtained results. The neglect of quantum effects is usually insignificant in biosimulations because of the sufficiently high temperatures and the lack of collective motions that cannot be represented classically.

The summary of the application of molecular dynamics for studying the process of GI drug absorption shows that some valuable structural and compositional insights are already gained. This computational technique is extremely powerful to characterize intermolecular interactions and elementary steps of processes. The treatment of the liquid environment of the bioactive molecules is reasonable, almost always involving explicit solvent. The milieu conditions are appropriate but care should be taken, e.g. for simulating at body, not at room temperature, for taking into account the proper pH or ionic strength, etc. Still an issue is the lack of fully atomistic simulations and those covering longer times. In addition, larger and more complex models are needed for more realistic description of some processes.

The results from MD simulations may be combined with the outcome from several experimental techniques to gain more multi-facetted insight into the target properties of drug formulations. The predicted data for size and structure of the DDSs may be compared to those from NMR or nuclear imaging. The information about the intermolecular arrangement may be synchronized with that from small-angle scattering experiments and surface plasmon resonance measurements. On the other hand, theoretically calculated parameters from MD simulations may be used to tune and improve the performance of pharmacometric and GI mesoscopic models. Last, but not least, the information about intermolecular interactions obtained from molecular dynamics modelling may be employed to explain the outcome from *in vitro* bioactivity testing. The opposite is valid as well – the MD computational protocol may be refined based on results from bioactivity tests.

This overview reveals that molecular dynamics simulations are a powerful source of molecular-level knowledge about the processes involved in GI drug absorption and fit well into the advanced methods toolbox.

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3. Dynamic simulation of pH fluctuation along the GI tract: the physiolution approach

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- Variation of pH level, amongst other factors, defines drug GI absorption and performance. Accordingly, methods to simulate lumenal pH are of a great value during drug development.
- For simulations, the importance of the nature of the buffer used, its capacity and ionic strength are discussed, as well as influence of volume, mechanical stress and GI fluid composition.
- Relevance of the different sections of the GI tract are stressed.

Summary

The pursuit of finding the best solution to reliably predict *in vitro* drug performance continues, mainly due to the high complexity that governs gastrointestinal (GI) conditions, that are in turn subject to high variation amongst human population. This section is focused on discussing how pH fluctuations define drug dissolution and summarises progress with existing technologies to mimic lumenal conditions and suggest possible future adjustments in the field. The use of telemetric techniques such as SmartPill® or IntelliCap® have significantly contributed to the understanding of pH variation along the GI tract. Choosing the appropriate buffer is the next fundamental step *in vitro* dissolution studies. Introducing bicarbonate buffer, instead of phosphate buffer, might result in better biorelevant prediction of drug dissolution behaviour in the pH range 5.5–8.4, with respect to the actual volume, buffer capacity and ionic strength. However, the creation of real-time high-resolution pH gradients combining biorelevant aspect of bicarbonate buffer, simulation of mechanical stresses and the composition of intestinal milieu are still not simulated with the existing devices. The solution to this problem would undoubtedly play an important role, not only in the dissolution studies, but also in understanding drug permeation considerations.

3.1. Introduction

The realistic estimation of the dissolution and absorption of the ionizable compounds in the gastrointestinal tract (GI) requires accurate simulation of the pH value and composition of the GI fluids. The pH value of the GI liquids is predetermined by secretion of several chemical species including the hydrochloric acid in the stomach; hydrogen carbonate salts in the small intestine; short-chain fatty acids and carbon dioxide resulting from the metabolic activity of bacteria in the gut. Therefore, a realistic simulation of pH fluctuations along the GI tract is challenging owing to the wide span of the pH values to be covered and the dynamic nature of the pH fluctuations. From the physiological perspective, the pH profiles have been well characterized using telemetric techniques such as IntelliCap® and SmartPill® (Koziolek et al., 2014) and (Koziolek et al., 2015a). Although well understood, the realistic simulation of the physiological pH fluctuations still represents a challenge. From the experimental perspective, a physiologically-orientated simulation of pH requires both liquid and gaseous titrants. Second, acidification and alkalization of dissolution media with a high-resolution pH gradient demands continuous and dynamic adjustment. For this purpose, we developed the pHysio-grad® - a novel device that enables a biorelevant simulation of gastric and intestinal pH gradients, and therefore the estimation of dissolution and drug delivery performance of oral medicines containing ionisable compounds.

In this section, we describe the use of the new generation dynamic pH controller - pHysio-grad® - for simulation of the physiological fluctuation of the pH along the GI tract using liquid and gaseous titrants. We also present the use of the device for the simulation of high-resolution pH profiles and provide examples on practical uses of the technique to predict the biopharmaceutical properties of the oral medicines and drug absorption.

3.2. Methods for simulations of the GI pH

Due to the complexity of the gastrointestinal (GI) tract, an oral dosage form is exposed to various physicochemical factors, which change sequentially along the gut regions. These are highly variable for different parts of the tract, and may have significant impact on drug delivery performance, particularly if the drug is a weak electrolyte. One of the major factors is the pH of the fluids, which might range from 1 to 8 along the GI tract. Physiological pH profiles along the tracts of adults have been intensively studied using telemetric capsules and pH-metric catheters (Fallingborg, 1999; Koziolek et al., 2015b,a; Schneider et al., 2019), the former also confirming considerable differences in transit times between individuals. Real time readings from telemetric measurements using SmartPill® (Schneider et al., 2016) or IntelliCap® (Koziolek et al., 2015a) have shown that these changes are fast and non-linear.

The pH profiles experienced by the dosage form along the GI tract depend on the prandial conditions and on individual characteristics of the individuals, such as disease, treatment, and diet. In the case of fasting intake, the initial pH of the gastric fluids ranges between pH 2 and 4 and remains nearly constant until the gastric emptying occurs (typically 0.25 - 2 h). In contrast, under standard fed intake conditions the initial pH at the intake of the dosage form ranges between 4.0 - 5.5 and decreases gradually to approximately pH 2 within about 3 h (Koziolek et al., 2015b).

The exposure to drug formulations to gastric conditions depend mainly on their size, intragastric mixing, drinking and the schedule of eating. Given the variability in gastric emptying according to the feeding regimen, the period of exposure can last from 2 to 20 h (Wilson et al., 1989). The sudden increase in the pH occurring after gastric emptying may lead to precipitation of the drug and an altered absorption process especially in the case where the drug is a weak base (Bevernage et al., 2012). The initial value of the intestinal pH ranges between 5.5 and 6.5

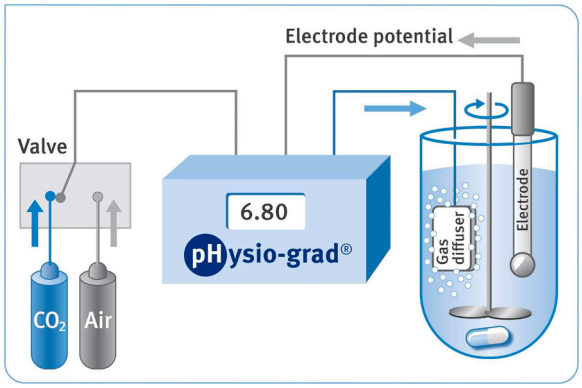

Fig. 3.1. The pHysio-grad® system [adapted from (Zakowiecki et al., 2020)].

for the small intestine and gradually increases (with a rate of up to 0.2 pH units per minute) in the distal direction up to pH 7.2 - 8.3 in the terminal ileum (Koziolek et al., 2015a). In contrast, the colonic passage is characterized by the presence of extremely dynamic fluctuations of the pH of up to Δ 2 pH units per 5 min in the pH range of 5.3 - 8.3. The transit times and the shape of the pH profile are intensively reviewed elsewhere (Koziolek et al., 2015a; Schneider et al., 2016 and Koziolek et al., 2015b). Other relevant factors, which affect pH profiles, and thus drug absorption and stability, such as buffer capacity and composition of the GI fluids have been described by other authors (Litou et al., 2016).

3.2.1. Method description

Executing physiological pH gradients experimentally using standard dissolution media, such as phosphate buffer, might be problematic because pH change occurs after the addition of a pH active reagent, an acid or a base). This, in consequence, leads to the change of dissolution volume and its ionic strength. Moreover, the effectiveness of pH adjustment is correlated with the concentration of titrants, so it might be difficult to ensure adequate precision. Manual executing of gradients in multiple vessels is laborious and time-consuming. Thus, without advanced devices, only low-resolution programmes can be reproduced in laboratory models.

The physiological alternative for phosphate buffer mimicking the intestinal milieu are the carbonic -hydrogen carbonate buffers. In the human body, bicarbonate is secreted by the Brunner's glands located in the mucosa and submucosa of the duodenum and the epithelial cells of the pancreas, to neutralize gastric acid (Krieg et al., 2015). This represents the main buffering system in the fasted state and enables the pH adjustment of chyme under postprandial conditions. Due to the abundance of HCO_3^- in the small intestine, the carbonic acid-bicarbonate mixture is considered the buffer system most biorelevant, experimentally. The pH of bicarbonate buffers ranges from 5.5 to 8.4, which matches the intraluminal pH conditions (5 - 8.4) (Garbacz et al., 2009). Moreover, the use of a buffer based on bicarbonate, due also to its buffer capacity, is comparable with the intestinal fluid properties *in vivo* (Litou et al., 2016, 2020; Hens et al., 2017).

Hydrogen carbonate buffer is a complex system characterized by thermodynamic instability. The pH value of the HCO_3^- buffer is a result of dynamic interplay between the amount of dissolved CO_2, the HCO_3^- ion concentration, and the partial pressure of the CO_2 in the atmosphere above the solution (Eq. 1). Both processes: alkalization and acidification are reversible (Garbacz et al., 2014; McNamara et al., 2003)

$$CO_2(gas) \rightleftarrows CO_2(aq) + H_2OCO_2(aq) + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

Eq. 1. The equilibrium equation of carbon dioxide solutions[8].

The use of hydrogen carbonate buffer with full control of pH value is provided by devices such as the pHysio-grad® system (Fig. 3.1) and the Auto pH System™ (Goyanes et al., 2015). They offer the possibility of continuous monitoring and pH adjustment. In both systems,

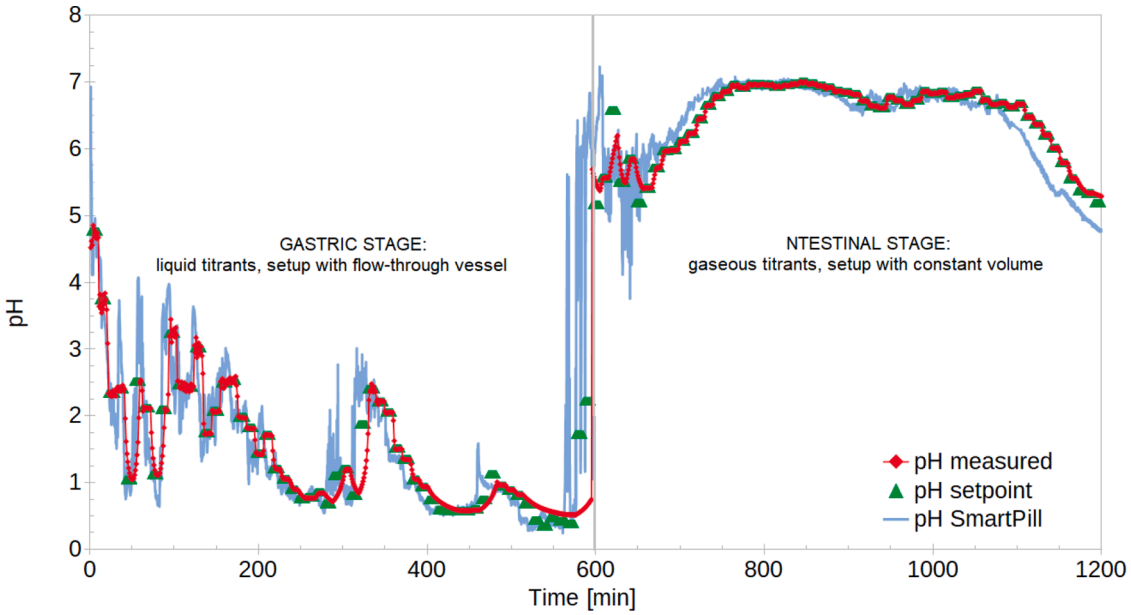

Fig. 3.2. Simulation of pH gradient in the GI tract under fed intake: pH gradient obtained during clinical trials using SmartPill® (blue line), pHysio-grad® input function (green points) and the actual pH measurement in the vessel (red points). Simulation of gastric environment was performed using liquid titrants (0.1 M HCl and 0.1 M NaOH) in a flow-through vessel. Simulation of intestine environment was performed using gaseous titrants (CO2 and air) in a vessel. SmartPill data kindly shared by (Koziolek et al., 2015a). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

acidification is obtained by purging CO_2 into dissolution medium. To reverse the acid drive, application of an inert gas such as compressed air, N_2 or He, leads to an enlargement of the surface of the solution resulting in acceleration of CO_2 evacuation from the medium and, in consequence, to the increase of pH. Such approach enables either maintaining constant pH or performing pH gradients reflecting changes in the intestinal part of GI tract. It is worth noting that, unlike other buffers, performing a pH gradient in a bicarbonate buffer does not involve a change in the volume and ionic strength of the solution, which is another important feature of this system.

Biorelevant *in vitro* dissolution testing can be used for prediction of *in vivo* performance of a drug product and allows limiting time consuming and expensive clinical studies, which is desirable from economical and ethical point of view. An increased discriminatory power of tests performed in hydrogen carbonate buffers has been described [Goyanes et al., 2015; Jede et al., 2019, Garbacz et al., 2015). Introduction of real-time pH gradient might be crucial for evaluation of some specific groups of drugs. This approach has been beneficial for modified-released (Zakowiecki et al., 2020; Karkossa et al., 2018), locally acting (Crowe et al., 2019), pH-sensitive or pH-triggered formulations (Garbacz et al., 2014). Up to date, only low-resolution pH gradients have been tested. It seems reasonable to simulate more complex, physiological gradients using dynamic pH controllers. However, there are some limitations. First, the use of hydrogen carbonate buffer limits the simulation to the intestinal part of the GI tract, as its pH range is within 5.5 to 8.4. It should be emphasized, that the point-by-point mimicking of the physiological pH gradients characterized by high resolution, as shown in the experiment with the SmartPill®, where the pH measurement took place every 5 s may be misleading. This inaccuracy is due to measurement artefacts which report high fluctuations of the pH along the GI. Therefore, results obtained from telemetric capsules must be first subjected to numerical processing in order to obtain simpler input data. At the same time, it would be important not to miss significant phenomena, such as sudden pH changes resulting from dynamically changing composition of intestinal milieu caused by secretion, motion or metabolism, or from intermittent contact of capsule with the intestinal fluid. In our estimates, only pH fluctuations lasting longer than 1 min are relevant for oral drug behaviour throughout the GI tract. Whilst this treatment simplifies the data, it should be appreciated that other parameters might be confounding; for example, that prolonged transit times could result from capsule's size. Moreover, the capsule's intrinsic properties might be the source of spontaneous measurement errors, such as drift of pH sensor or temporary signal loss. This in fact is likely to be observed because of variations in intestinal conditions. Sudden pH changes require tremendous gas flow at an elevated pressure, or higher concentrations of titrants, resulting in a more dynamically changing simulation, but lacking precision in the episodes of constant pH. In the case of hydrogen carbonate buffer, optimal accuracy and precision can be reached by an adequate device control setup.

Simulation of real-time high-resolution intestinal pH gradients, based on SmartPill® data, using a pHysio-grad® device, has been satisfactorily performed (Fig. 2). However, deviations occurred during simulation of the gastric environment. Based on the telemetric studies, the pH value in the fed stomach after ingestion of SmartPill® was initially in the range 3.3–5.3 and then slowly decreased to pH 1 in about 4 h (Schneider et al., 2016; Koziolek et al., 2015b,a). Such a low pH cannot be simulated by a carbonate buffer, and as consequence, other mediums and highly concentrated liquid titrants, such as HCl and NaOH or $NaHCO_3$ solutions are required. First attempts to perform such simulations, in a standard dissolution vessel, were unsatisfactory due to the high consumption of liquid titrants. This caused significant increase in the volume and the ionic strength of the medium. Consequently, such experiments should be performed in a flow-through setup. This would ensure better performance of the tests at physiological volumes, and would reflect realistic pH change kinetics and would result in more dynamic and accurate simulations. The flow-through method is also more relevant from the physiological point of view, and more convenient especially for drugs characterized by low solubility to maintain sink conditions.

3.3. Conclusion

To improve our understanding of various processes, which in completion lead to the final physiological effect of administered medicine, different scientific approaches are implemented. One of them is focused on the huge role played by mechanical stress, resulting from the

motility in the GI tract, on dissolution behaviour of an oral dosage form. Innovatory equipment simulating peristaltic action of the stomach and intestines seems even more reasonable, especially when combined with biorelevant dissolution media. Since bicarbonate buffer is found to be the closest reflecting intestinal fluid, its use in these novel devices might be of particular interest. Such approach was implemented in the study of mesalazine dissolution with the Stress Test device, and with Hanks' buffer as an intestinal medium, including the simulation of peristaltic action (Garbacz et al., 2015). In this case however, bicarbonate buffer was maintained at a constant pH value by the use of pHysio-stat®, a situation that does not reflect the variations experienced during intestinal passage. Realistic pH fluctuations could be ensured by the dynamic pH-controller and such improvement in the experimental setup would contribute to more predictive description of drug's dissolution performance *in vivo*.

In case of poorly soluble, but highly permeable drugs, we also perceive the dissolution/permeation devices as an opportunity to make use of the high-resolution pH-controllers. Several studies proved the importance of pH shift from the acidic to the neutral conditions and the outcome of such procedure in the following measurement of drug absorption rate (Borbás et al., 2018; Li et al., 2018; Miki et al., 2017). It is worth considering also the context of the supersaturation and precipitation properties of poorly soluble substances, both playing important roles during the transit of drugs through the GI tract.

The simulation of high-resolution pH gradients requires a thorough investigation of available *in vivo* data and development of a dedicated experimental setup. Technical issues when performing dynamic pH adjustment with acceptable precision, with physiological volumes and flow rates are not trivial. However, addressing these issues might result in development of a valuable *in vitro* tool, which might be used not only in advanced, bio-predictive dissolution testing, but also it can be integrated with other studies, including permeation or/and simulation of mechanical stresses. Several approaches to better understand the extensive and complicated drug performance of oral dosage forms through the human gut have been made, but there is still plenty of space for future approaches in order to improve the prediction of the drug dissolution of oral medicines.

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4. The BioGIT model for evaluating the impact of gastrointestinal drug transfer process on luminal drug product performance and early exposure in the fasted state

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- BioGIT has been shown to be useful in evaluating the impact of dose and the impact of formulation on luminal product performance and early exposure of lipophilic weak bases, enabling products, and complexes with ion exchange resins in the fasted state
- Aqueous solutions, aqueous suspensions, disintegrating products, or dispersing products (to be) administered with glass of water to healthy young adults can be studied with BioGIT
- Based on data collected to date, BioGIT should be used cautiously, in cases of substantial deviations from the above-mentioned conditions

Summary

The biorelevant gastrointestinal transfer (BioGIT) model is an open, three-compartment *in vitro* apparatus simulating the drug transfer process from the stomach through the upper small intestine, after oral administration of aqueous solution, aqueous suspension, disintegrating drug product or dispersing drug product dosed to fasted young healthy adults with a glass of water. Based on the achieved drug concentrations in the duodenal compartment, the influence of formulation and the impact of dose on product performance in the upper GI lumen and on early exposure can be evaluated.

4.1. Introduction

Although there may be situations in which drug absorption from the stomach can occur, as evident in dogs who were dosed after pyloric ligation and shown to absorb semaglutide (Buckley et al., 2018), in general, the GI (GI) drug transfer process from stomach to intestine is clinically important. This can be illustrated in the following cases.

For drugs with minimal intestinal permeability limitations which are rapidly dissolving or have high solubility in the upper small intestine, e.g. weak acids with low pKa, the onset and rate of appearance into the general circulation is governed by gastric emptying rates (e.g. Yazdanian et al., 2004). For enteric-coated products, initiation of drug release (and subsequently absorption) is, by definition, dependant on the characteristics of transfer from the stomach into the upper small intestine. In these two situations, the impact of GI transfer on the luminal performance of a given product could be evaluated by considering the dissolution characteristics under conditions, which separately simulate the gastric environment and the environment in the upper small intestine (Butler et al., 2019).

The third instance to consider is when the drug is administered as an enabling product. The drug achieves concentrations above the thermodynamic solubility for a transient time by formulation in a supersaturating environment, although this time may be too short for practical use (Augustijns and Brewster, 2012). In 2016, the majority of compounds under development were identified as low solubility / high permeability active pharmaceutical ingredients, with characteristics such as high crystal lattice energy, low aqueous solubility, poor dissolution rates and high molecular weight (Taylor and Zhang, 2016). This situation has been proposed to occur through advances in high throughput screening techniques and the development of novel screening methods used for the identification of druggable targets. These protocols promote active ingredients with therapeutic potential that exhibit increased molecular weight and octanol–water partition coefficient and lower aqueous solubility outside of Lipinski's rule of five (Lipinski, 2000, 2002; Price et al., 2019). To mitigate the solubility limitations of many new drug candidates, one increasingly common approach has been to achieve and maintain supersaturated concentrations of drug in the GI lumen during the absorption process, i.e. to develop enabling drug formulations.

A fourth situation where the GI transfer process determines drug absorption rates is when the drug does not have major intestinal permeability restrictions and has high solubility in stomach but low solubility in the upper small intestine, e.g. in case of a lipophilic weak base. Weak bases comprise the majority of orally administered APIs (Paulekuhn et al., 2007).

In the latter two situations, drug availability in molecularly dispersed state, i.e. in a state that allows for its transport through the intestinal epithelium, does not depend only on dissolution efficiency but, in addition, on its ability to achieve and/or maintain supersaturation (a thermodynamically unstable condition) and avoid fast precipitation in the contents of upper small intestine. As a result, in these two cases, the impact of drug GI transfer relies on luminal product performance which suggests the application of more sophisticated *in vitro* methodologies simulating the GI process, with the radial gradient of disappearance from the lumen (due to absorption) as much as possible (O'Dwyer et al., 2019; Butler et al., 2019).

It was well appreciated by industry that *in vitro* assessments based on compendial dissolution apparatus of different types had limitations, particularly with regard to devising more accurate biorelevant tools rather than a highly standardised batch quality control system (Butler

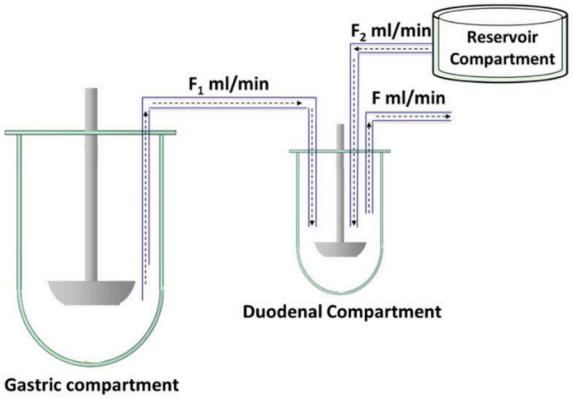

Fig. 4.1. Schematic representation of the BioGIT model. F_1 and F_2 are the incoming flow rates and F is the outgoing flow rate; $F = F_1 + F_2$. [Kourentas et al., 2016a].

et al., 2019). The early attempts at a simulator with attention to the mechanical forces, designed on the basis of measured human physiological parameters, TIM-1, proved successful in the nutrition and pharmaceutical industries (Blanquet et al., 2004) but had several limitations in terms of complexity and cost and few academic centres had access to such devices. Accordingly, need was fed by innovation and the EU initiative OrBiTo, composed of contributions of industry and academic scientists sifted through the available tools in existence or development in the field of biorelevant dissolution methodology (Kostewicz et al., 2014). In this paper, the principle features of BioGit were described, alongside adapted pharmacopeial methods and other innovations. The OrBiTo and UNGAP programmes enabled further development and more applications, which are described in this section of the review.

4.2. The BioGIT model and drug absorption

The BioGIT model is an open three-compartment *in vitro* set-up simulating the drug transfer process after oral administration of certain dosage forms from the fasted stomach through the upper small intestine (Fig. 4.1) (Kourentas et al., 2016a). The design has been based on luminal data collected after intragastric administration of 240 ml aqueous solutions of single oral doses of two lipophilic weak bases, ketoconazole (100 mg and 300 mg) and dipyridamole (30 mg and 90 mg), to healthy fasted adults (Psachoulias et al., 2011). Similarly, *In vitro* testing conditions were based on volume of duodenal contents and drug input/output duodenal rates estimated, after modelling luminal data of highly permeable drugs (Psachoulias et al., 2011). In this protocol, the conditions in the duodenal compartment take into account both the transport of a highly permeable drug via the epithelium of upper small intestine and the transit along the lumen of upper small intestine. In case of the solution formulation, the% precipitated in upper small intestine is estimated. Using a part of the sample collected from the duodenal compartment for measuring apparent drug concentration, apparent equilibrium solubility can be measured and subsequently apparent supersaturation in upper small intestine can be estimated.

In certain situations, such as when conventional products of lipophilic weak bases and enabling drug products are considered, the product performance in upper GI lumen can limit oral drug absorption. In those situations, investigation of product performance in upper GI lumen is relevant to the study of oral drug absorption. BioGIT has been proposed for the investigation of the impact of dose and of formulation on the performance of conventional products of lipophilic weak bases, and of enabling drug products in the upper GI lumen.

Since the design of BioGIT has been based on drug presence in the upper small intestine of adults as function of time after drug administration, the presence of drug in the duodenal compartment of BioGIT simulates the net output of three processes that occur simultaneously, i. e. the gastric emptying process, the transport of drug through the intestinal mucosa, and the transit along the upper small intestine. Following this appreciation, BioGIT has also been proposed for the evaluation of the impact of formulation and the impact of dose of highly permeable drugs on early exposure.

4.2.1. Usefulness of BioGIT model

When measurements are performed, comparing luminal data in the adult gut to samples obtained on timed sampling in the apparatus, the BioGIT model appears to be a useful tool allowing estimation of the apparent drug concentrations and% solid fraction in the upper small intestine, for example after administration of a drug in solution or suspension, and after administration of disintegrating/dispersing dosage form with a glass of water to fasted young adults.

Additionally, following evaluations of early exposure data in adults, the BioGT model appears to be useful in predicting the impact of dose and of formulation on early exposure. Over time, strengths and limitations of the technique have become apparent. Specifically, in pharmaceutical research and development, the BioGIT model could be useful for the evaluation of:

- The study of the impact of dose and formulation on early exposure, for example after oral administration of disintegrating/dispersing/solution dosage forms (conventional or enabling) with a glass of water to healthy adults (Kourentas et al., 2018).
- The estimation of luminal concentrations in the upper intestinal lumen during the first hour of the absorption process after administration as above to healthy adults with normal gastric acid secretion rates (Hens et al., 2014; Kourentas et al., 2016b; van den Abeele et al., 2020) or under hypochlorhydric conditions (van den Abeele et al., 2020).
- The measurement of the precipitated fraction in upper intestinal lumen during the first hour of the absorption process (Kourentas et al., 2016c).
- The assessment of the performance of drug-ion exchange resin complexes in the upper GI lumen, after oral administration to fasted adults with a glass of water (Yamamoto et al., 2020).

The BioGIT model could also be useful in the regulatory setting, e.g.

- in providing supporting information on the impact of dose and formulation on early exposure, after oral administration of disintegrating dose units, suspensions or solutions in the fasted state (Kourentas et al., 2018), and
- for informing physiologically based biopharmaceutics (PBB) models on precipitation kinetics in the upper small intestine (Kesisoglou et al., 2018).

It should be underlined that the BioGIT model, to date, has been shown to be useful to guide selection of formulation and evaluate the impact of dose on apparent drug concentrations in upper small intestine early after drug administration on a qualitative basis. A procedure for using the BioGIT model on quantitative basis has been proposed (see Fig. 5 in Kourentas et al., 2018);however, more case studies are needed to confirm its usefulness.

Although the design of the BioGIT model has been based on luminal data of highly permeable compounds (Kourentas et al., 2016a), in some of the above situations, low permeability compounds have been used (Hens et al., 2014; van den Abeele et al. 2020). Slight overestimation of duodenal concentrations, very early after drug administration, was observed in those situations (Hens et al. 2014; van den Abeele et al. 2020).

It should be acknowledged that other models have also been proposed to evaluate the impact of GI drug transfer process on drug disposition after oral administration in the fasted state. The artificial stomach-duodenum (ASD) model is, as BioGIT, an open system (Carino

et al., 2006). The so-called "transfer model" is a closed system (contents of gastric compartment are accumulated in the intestinal compartment (Kostewicz et al., 2004). The transfer model is primarily used for evaluating the tendency of precipitation in the small intestine and it has been useful in the development of PBB models (Ruff et al., 2017; Pathak et al., 2017). However, for both the ASD and the transfer models published information on their usefulness in estimating the parameters they have been designed to estimate, against human data, is very limited, to date.

4.2.2. Limitations of the BioGIT model

The BioGIT model has been shown to work as a screening tool for assessing concentrations in upper small intestine the impact of dose and formulations on product performance and on in early exposure, after administration of disintegrating solid or liquid dose units with a glass of water to young adults with normal gastric acid secretion rates on a crossover basis. Also, ideally for highly permeable APIs. If the dose units tested *in vitro* are not identical with those tested *in vivo*, or if *in vivo* data have not been collected in healthy adults with normal gastric acid secretion rates, or if administration to healthy adults has not been performed with a glass of water, the BioGIT model should be used cautiously. Substantial deviation(s) from the "young adults with normal gastric secretion rates" requirement or the "glass of water" may lead to random gastric emptying *in vivo* (in case drug administration is performed with less than about 250 ml water) and/or substantial modification of the gastric emptying process (in case co-medications are received by patients).

4.3. Conclusions

In this section the BioGIT model for evaluating the impact of GI drug transfer process on drug disposition in the upper GI lumen, after oral administration, was presented. Data collected to date suggest that BioGIT is useful in the evaluation of the impact of dose and of the formulation on luminal product performance and/or early exposure of solutions/suspensions/disintegrating conventional or enabling drug products, and of complexes with ion exchange resins after administration to young healthy adults with a glass of water in the fasted state.

5. Simulating the stomach and upper small intestine: the GastroDuo model

By Mirko Koziolek, and Philipp Schick. NCE Formulation Sciences, Abbvie Deutschland GmbH & Co. KG, and Department of Biopharmaceutics and Pharmaceutical Technology, center of Drug Absorption and Transport, University of Greifswald, Germany.

- The GastroDuo is a dynamic *in vitro* method to evaluate how physiological aspects of the upper GI tract affect drug release of IR dosage forms under fasted and fed state conditions
- This biorelevant *in vitro* tool can simulate physiological pressure events, gastric emptying rates and peristaltic movements occurring in the human stomach. Moreover, it also enable the simulation of realistic pH and temperature profiles in the upper GI tract.
- The simulation of rapid emptying of non-caloric fluids (*Magenstrasse*) can also be performed to evaluate how this phenomenon affects the transfer of dissolved and undissolved drug from the stomach into the small intestine.
- Dynamic UV-measurements provide information on whether drugs are emptied from the gastric compartment as a solution or as small, solid particles. By this, this device provides further mechanistic insights into formulation performance in the GI tract.
- The application of GastroDuo to understand human data for different drugs is discussed to highlight the importance of *in vitro* methods to support formulation development and optimisation with respect to the release properties of oral formulations

Summary

Biorelevant dissolution testing is a key instrument during the development of novel drug formulations as the application of physiologically relevant *in vitro* drug release models allows to better understand the effect of physiological parameters on *in vivo* disintegration and drug dissolution and thus, also on the resulting drug plasma levels. However, the physiological conditions are highly complex and dynamic, which makes it extremely difficult and expensive to cover all parameters of potential relevance in a single experiment. The GastroDuo, which is described in this section, represents an *in vitro* model that focusses on the abstract and reproducible simulation of specific physiological parameters in order to study their impact on drug product performance in a rational manner.

5.1. Introduction

The stomach is of great importance for the disintegration and release behaviour of oral immediate release (IR) formulations (Vertzoni et al. 2019). Therefore, the conditions inside the stomach as well as the process of gastric emptying can strongly affect the pharmacokinetic profile of oral drug products. Since relevant physiological parameters such as gastric pH, gastric emptying kinetics or gastric motility show high intra-

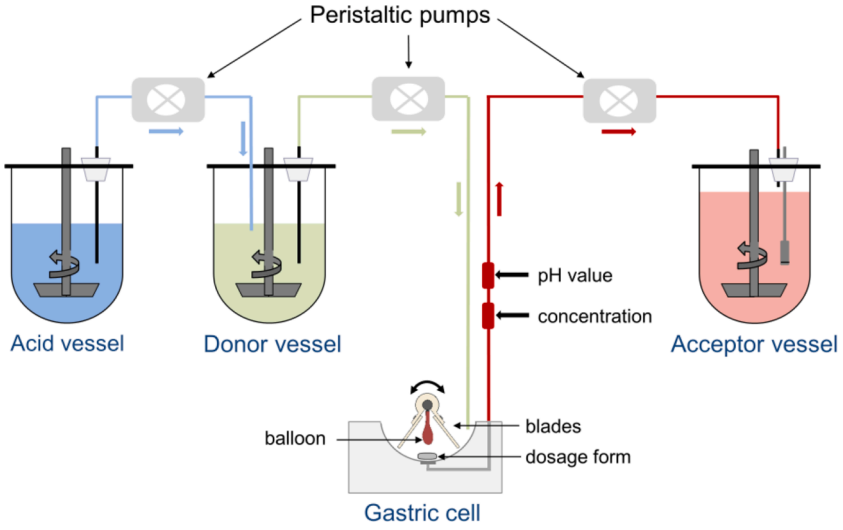

Fig. 5.1. Schematic illustration of the GastroDuo.

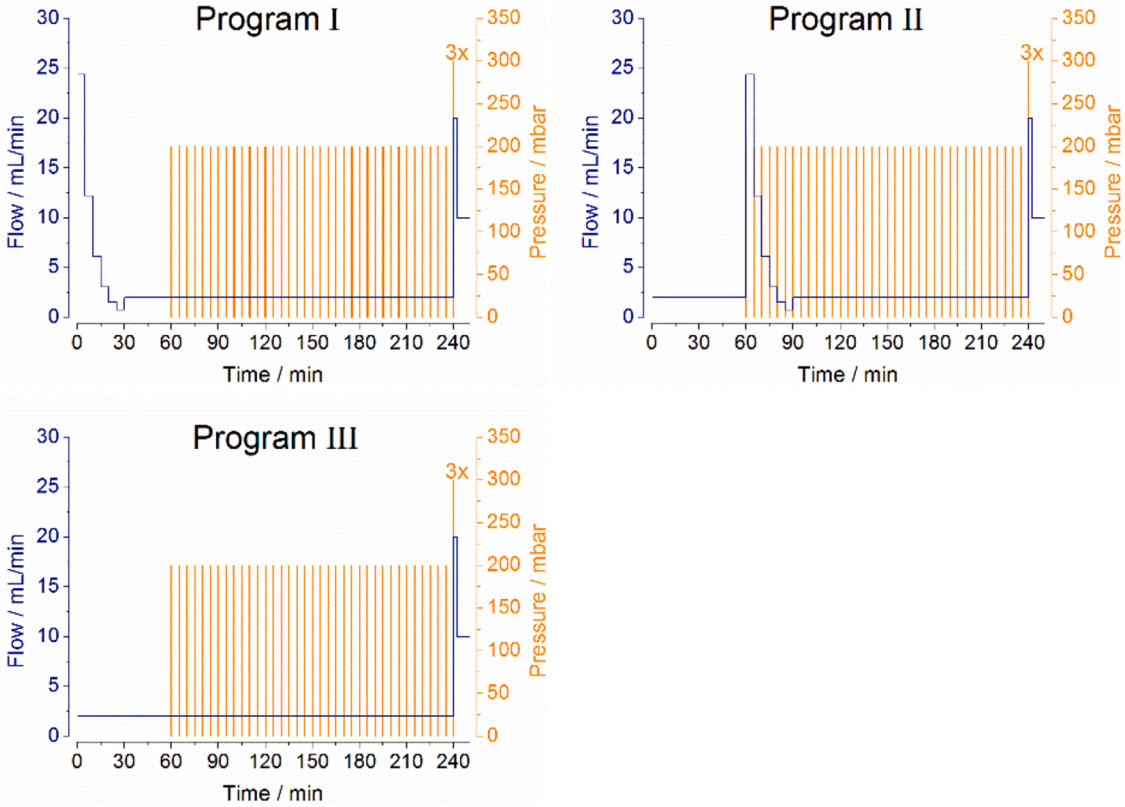

Fig. 5.2. Test programs applied in the GastroDuo to simulate different gastric emptying patterns present in the fed state. Blue lines indicate flow rate and orange bars pressure events. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and interindividual variability, they also contribute to the variability of plasma drug profiles (Vinarov et al., 2021). This variability can be minimized by suitable oral formulations, but for their development, appropriate *in vitro* models must be available. Despite the tremendous efforts in developing new biorelevant *in vitro* methods, the variability of physiological factors is often not considered in these methods. Therefore, it is still extremely challenging to develop formulations that are robust against variations of the physiological conditions or that are able to specifically exploit physiological parameters for drug release. Hence, there is a clear need for *in vitro* methods, which allow a physiologically relevant characterization of formulation candidates in order to enable targeted and effective formulation development already at early stages.

5.2. The GastroDuo

Recently, the GastroDuo was introduced as an *in vitro* device, which enables a physiologically relevant simulation of gastric transit of oral drug products in an abstract and reproducible manner (Schick et al., al., 2019; Sager et al., 2019a; Schick et al., 2020). This device is a transfer system with four compartments, which are arranged in the following order: acid vessel, donor vessel, gastric cell, and acceptor vessel (Fig. 5.1).

For formulation testing with the GastroDuo, which can be performed in triplicate, the dosage form to be tested is placed inside the gastric cell between two blades. These blades are mounted on a central axis connected to a stepping motor. By this, it is possible to simulate movements of the dosage form as they were observed by Magnetic Marker Monitoring inside the human stomach (Weitschies et al., 2005; Koziolek et al., 2014). A balloon located at the axis between the blades can be inflated by compressed air and thus, physiologically relevant pressure events can be simulated. Their magnitude is based on data generated recently by using the SmartPill™ in healthy volunteers (Koziolek et al., 2015a). The GastroDuo is designed as a flow-through system, in which several individually controllable peristaltic pumps allow the transfer of media. The gastric cell is perfused with medium from the donor vessel, in which the conditions can be modulated as well. This is accomplished by pumping contents from an acid vessel. Thus, pH and temperature profiles can be generated as they were recently measured by SmartPill™ (Koziolek et al., 2015a; Schneider et al., 2016). The outlet of the gastric cell is located at the bottom. This allows the transfer of particles smaller than 2 mm. Directly after the outlet, an in-line measurement for pH and drug concentration is enabled by pH electrodes and UV–Vis fibre optic dip probes. In this way, it is possible to determine drug concentration profiles as they are expected to occur in the duodenum. This provides also the information whether drugs are emptied in dissolved state or as small solid particles. This information can be highly relevant to better understand the onset of drug plasma concentrations of certain drug products. In the case of drugs, for which the rate of absorption into systemic circulation depends on luminal drug concentration (e.g. in case of active transport or intestinal metabolism), the question in which form the drug will be emptied is highly relevant. The overall transferred medium (and drug) is finally collected in an acceptor vessel, where drug concentration is also quantified photometrically by UV–Vis fibre optic dip probes. Based on this information, a mass balance profile can be obtained that enables the determination of the remaining amount of drug inside the gastric cell. Moreover, together with potential peaks in drug concentrations measured at the outlet of the gastric cell, it is also possible to assess the effect of different simulated parameters on drug release from the formulation.

5.2.1. Experimental approach of the GastroDuo

To test the sensitivity of oral formulations towards variations of certain physiological parameters known to be critical for drug release in fasted and fed state (e.g. gastric pH, gastric emptying kinetics, gastric motility, etc.), different test programs can be applied to simulate these parameters at different levels. In general, the impact of the complex

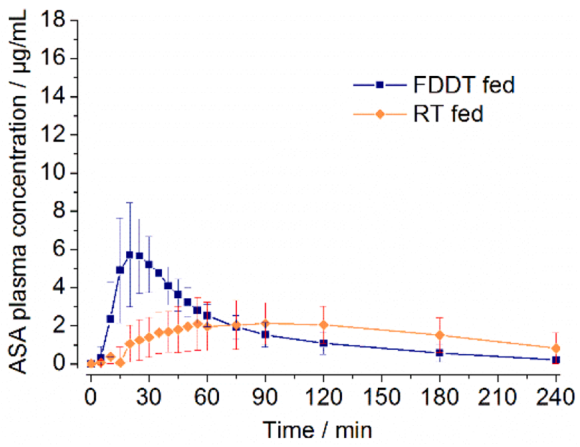

Fig. 5.3. Comparison of the pharmacokinetic mean profiles of the regular tablet (RT, means of $n = 29 \pm$ SD) and the fast disintegrating and dissolving tablet (FDDT, means of $n = 30 \pm$ SD). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

human GI physiology on drug release may also be studied in one experiment (e.g. Dynamic Gastric Model, TNO TIM-1 system), but in this case, it is almost impossible to draw meaningful conclusions about the contributions of individual parameters. One of these parameters is a physiological phenomenon referred to as stomach road or *Magenstrasse,* which describes the rapid emptying of non-caloric fluids from the postprandial stomach (e.g. water). Interestingly, this water is not mixed into the gastric chyme, but can bypass the stomach along the gastric walls and thus, may take a shortcut into the small intestine. The existence of this phenomenon is expected to have significant impact on the onset of drug plasma concentrations of many orally administered IR formulations (Grimm et al., 2017). To test how this physiological phenomenon affects drug release and thus indirectly drug absorption, different flow rate profiles can be simulated in dedicated GastroDuo programs (Fig. 5.2). Thereby, the flow rates ae adapted to simulate the effect of the *Magenstrasse*, either for the fluid co-administered during drug intake (program I) or for the fluid administered 1 h after drug intake (program II). In another program (program III), the presence of the *Magenstrasse* is not simulated and only a constant flow rate is applied to reflect the emptying rate of food from the stomach. Since it is expected that formulations are initially deposited in the fundus if administered after the intake of food, no pressure events are simulated in the first hour.

Recently, the GastroDuo was successfully used to obtain a mechanistic understanding of human data from two fed-state, pharmacokinetic studies for the approved drug products Viagra® and Adenuric®. (Schick et al., 2019). The resulting individual plasma levels of both drug products can be explained by their different release and emptying characteristics. The *in vitro* tests revealed that the disintegration and release behaviour of both drug products is dependant on the simulated hydrodynamic and mechanical effects. The pharmacokinetic data for Viagra® showed a slow and continuous plasma onset over several hours in most of the volunteers. This observation is confirmed by the *in vitro* results, which reveal an emptying behaviour that is strongly affected by the simulated pressure and movement events. Thus, without occurrence of mechanical stresses acting on the drug product, disintegration is delayed and consequently, this drug cannot be emptied via the stomach road. In contrast, a rapid plasma onset of drug plasma concentrations is observed for Adenuric® in most of the volunteers. This is also in accordance with the *in vitro* results, which show that the drug product is rapidly dispersed into fine particles and that the emptying of the drug into the acceptor vessel is mainly controlled by the rate of perfusion of the gastric cell. It can be expected that this drug is mainly emptied via the *Magenstrasse*. This study nicely illustrated how the disintegration and dissolution behaviour of oral drug products can affect the onset of drug plasma concentrations and how tools such as the GastroDuo could support the development of formulations with the desired PK profile. Similar observations were made recently for a novel Aspirin® formulation (Schick et al., 2020). Thanks to the rapidly disintegrating and dissolving character of this formulation, which goes back mainly to the addition of a strong disintegrant and the micronisation of the drug, the emptying of the drug from the fed stomach into the small intestine is clearly accelerated as compared to the previous regular tablet (RT) formulation (Fig. 5.3).

As was confirmed by the *in vitro* data obtained by use of the GastroDuo, the accelerated drug plasma onset of the FDDT is again based on the rapid emptying of the drug from the fed stomach via the *Magenstrasse*. In contrast, the regular tablet, which disintegrates slower and also into larger particles, is emptied slowly and incompletely from the gastric cells. Consequently, the onset of drug plasma concentration is also much slower *in vivo*, which results in lower values for C_{max}, as compared to the novel formulation. Again, this case study highlights the necessity of *in vitro* methods capable to represent physiologically relevant aspects to support development of formulations with optimized release properties.

Apart from the simulation of fed conditions, the GastroDuo can also be used to investigate drug disintegration and dissolution in fasted state (Sager et al., 2019a). In a recent work, Sager *et al.* studied the performance of different capsule shells (i.e. hard gelatine vs. HPMC based capsules) in comparison to a film-coated tablet. For this study, caffeine was chosen as a model drug since it dissolves quickly and therefore, the onset of *in vitro* concentrations is mainly controlled by disintegration. Moreover, caffeine can also be used as a marker for *in vivo* disintegration experiments (Sager et al., 2019b, 2019c). In this study, six test programs

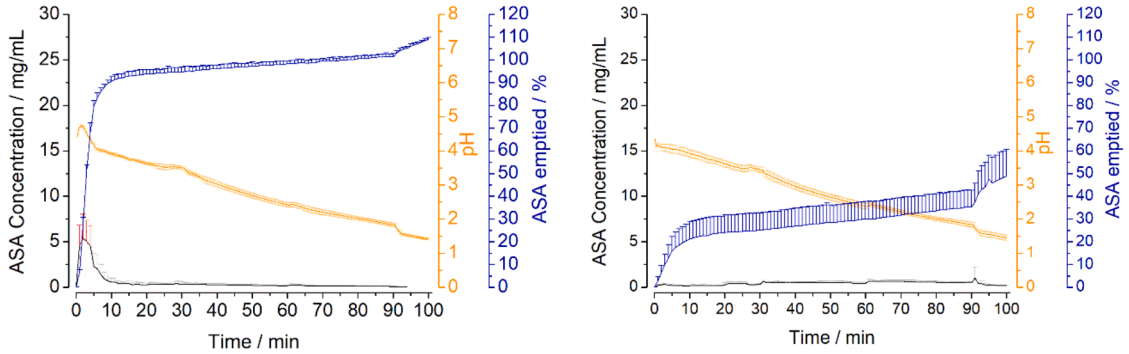

Fig. 5.4. Concentration (black) and pH profiles (orange) measured at the outlet of the gastric cell as well as the amount of ASA emptied into the acceptor vessel (blue) over time for two different Aspirin formulations (left: fast disintegrating and dissolving tablet, right: conventional tablet). Means of $n = 6 \pm$ SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reflecting different physiological aspects of the fasted stomach were used to evaluate the disintegration and dissolution behaviour of the different formulations. Subsequently, the same formulations were also administered to healthy, young volunteers to determine the *in vivo* disintegration times with the aid of the salivary tracer technique. In this way, it was possible to compare the *in vitro* data generated by the GastroDuo to the corresponding data from *in vivo* studies. The results showed that the GastroDuo was able to detect certain differences in the disintegration and dissolution behaviour of the dosage forms. However, not all *in vitro* observations in GastroDuo experiments were in accordance with the *in vivo* data. This effect is attributed to the fact that the simulated profiles of the physiological parameters can occur with different probabilities.

5.2.2. Application and limitations

The GastroDuo represents a promising tool for the evaluation of IR formulations that enables the reproducible simulation of physiologically relevant aspects. However, a series of experiments is required in this *in vitro* approach to evaluate in which rate and extent certain physiological parameters affect disintegration and drug dissolution. In this way, an evaluation of GI key parameters on the performance of oral drug products is possible. Due to the modular design of the GastroDuo, it is also possible to identify the most critical parameters in terms of drug release from a certain drug product.

As mentioned, it should be noted that this *in vitro* approach always requires a series of experiments. Moreover, it must be considered that the simulated programs are designed to describe the physiological range of certain parameters, but not all simulated levels are likely to occur *in vivo*, especially not in the controlled environment of a clinical study. Nonetheless, the ranges simulated for gastric emptying, gastric pH and temperature as well as gastric motility are based on recent *in vivo* data and therefore considered to be realistic for the majority of patients. Thus, to guarantee the efficacy and safety of a particular drug product in real life, drug release should be robust against any variation of these parameters.

Since not all physiological aspects of the human GI tract can be covered by the GastroDuo, the additional use of other well-suited models such as transfer model, BioGit or TNO TIM-1 is recommended to get deeper insight into how physiological parameters affect drug product performance. The combination with appropriate permeation models may also be useful, since the GastroDuo focusses only on investigating the processes of drug release and gastric emptying. By this, valid assumptions with regard to drug absorption can already be made, but these are limited to well absorbable drugs where processes like gastric emptying are rate limiting. However, in most cases only multi-assay approaches will allow formulators to get deeper insights into formulation performance in the human GI tract.

5.3. Conclusion

A profound evaluation of oral formulations already at early stages ultimately leads to the development of oral drug products that are robust towards the variable conditions in the human GI tract. If this is not the case, the safely and efficacy of oral drug products may not be given in real life. In this context, the GastroDuo can be a promising *in vitro* tool, which allows to simulate various parameters highly important for drug release in an abstract, but dynamic and reproducible manner. The experimental approach, which always involves a series of experiments, as well as the modular design of the GastroDuo allow formulators to study the impact of individual physiological parameters on drug release. Since the GastroDuo does not contain an absorption compartment, it is currently limited to formulations containing well absorbable drugs. For these, the processes of drug release and gastric emptying are mainly driving the absorption process. However, the GastroDuo may be combined with appropriate absorption models to get further insights towards the dynamic interplay of drug release and absorption.

6. *Ex vivo* use of aspirated human intestinal fluids to unravel drug absorption processes

By Tom de Waal, Joachim Brouwers, and Patrick Augustijns, KU Leuven, Belgium

- The section revises the importance and the synergetic value of applying aspirated human intestinal fluids in the *in vitro* simulation protocols revised before, to better understand the interaction between intraluminal factors and oral drug products. Methods to obtain intestinal fluids from different compartments in fasted and fed state, as well as the intraluminal dynamics with respect to fluid composition and ultrastructure are briefly described.
- The relevance of using human intestinal fluids *ex vivo* to identify critical intraluminal factors for drug absorption and to guide the optimisation of simulation tools and models is illustrated.
- Future challenges and opportunities of using human intestinal fluids are discussed

Summary

Simulated intestinal fluids are commonly used in the *in vitro* evaluation of intestinal drug absorption thanks to their simplicity, ease of use and cost efficiency. However, they do not encompass the sheer complexity and dynamic nature of human intestinal fluids. In certain scenarios, the use of simulated fluids falls short to predict drug absorption and understand underlying processes. For instance, the interplay between solubilisation, supersaturation, precipitation and permeation of lipophilic drugs in fed state conditions is hard to evaluate in commercially available simulated fluids, as those are lacking the complex ultrastructure of postprandial human intestinal fluids. In addition, the fixed composition of simulated fluids makes them unsuited to assess the sensitivity of oral drug products to the inherent compositional and functional variability of human intestinal fluids. Unravelling these scenarios therefore requires the *ex vivo* use of aspirated human intestinal fluids in absorption-related assays, which will eventually guide the optimization of biorelevant simulation media and tools.

6.1. Introduction

The GI absorption of drugs is the result of the complex interplay between the physicochemical properties of the drug, the formulation strategy employed, and the highly dynamic and complex intestinal environment. In this respect, the composition of intestinal fluids is widely recognized as a major actor in drug dissolution, precipitation and permeation. To predict the effects of *in vivo* relevant concentrations of bile salts, phospholipids, and fatty acids on these absorption-determining processes during the early stages of drug development, multiple simulated intestinal fluid compositions with different levels of complexity have been introduced (Markopoulos et al., 2015; Fuchs et al., 2015; Vertzoni et al., 2010). For obvious reasons, these biorelevant media are designed to be simple, relatively cheap, and easy to integrate in drug development. This implies however, that they encompass neither the full complexity nor the inherent variability of the dynamic human intestinal fluids. Although the existing simulated fluids are highly valuable to increase the biorelevance of dissolution and permeation testing, there are still scenarios where they fall short in providing adequate predictions of drug behaviour in the human intestine. The present section discusses the *ex vivo* use of aspirated human intestinal fluids in absorption-related assays as an advanced tool to unravel those scenarios and, ultimately, to guide the further optimization of biorelevant media.

6.2. Ex vivo use of aspirated human intestinal fluids in drug absorption assays

Human intestinal fluids are typically aspirated from either the

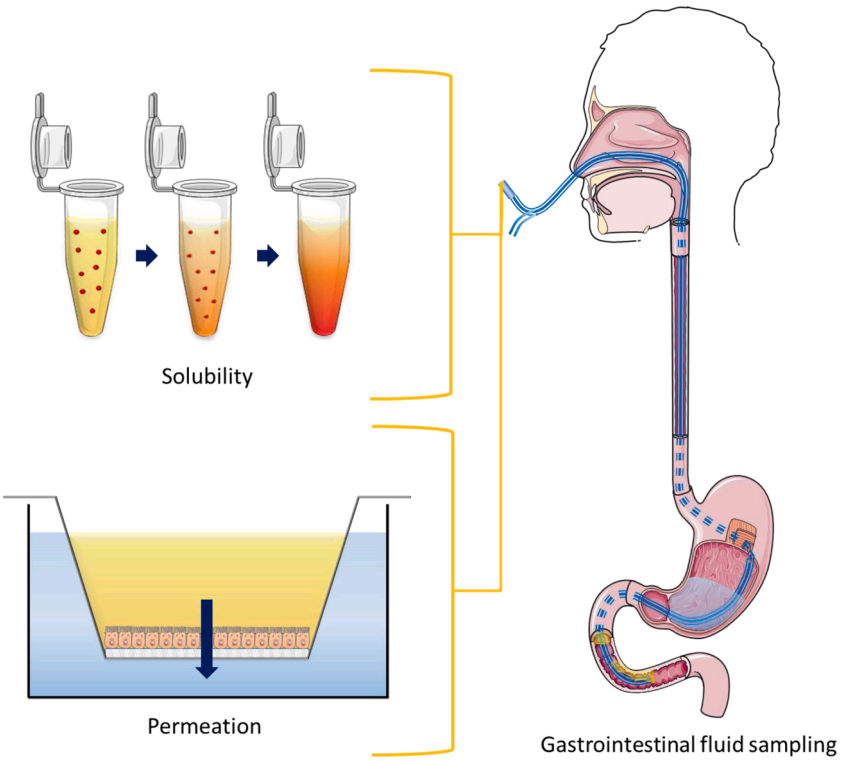

Fig. 6.1. Graphical representation of human intestinal fluid sampling and the use of these aspirated fluids in absorption-related assays as an advanced tool to unravel drug absorption processes.

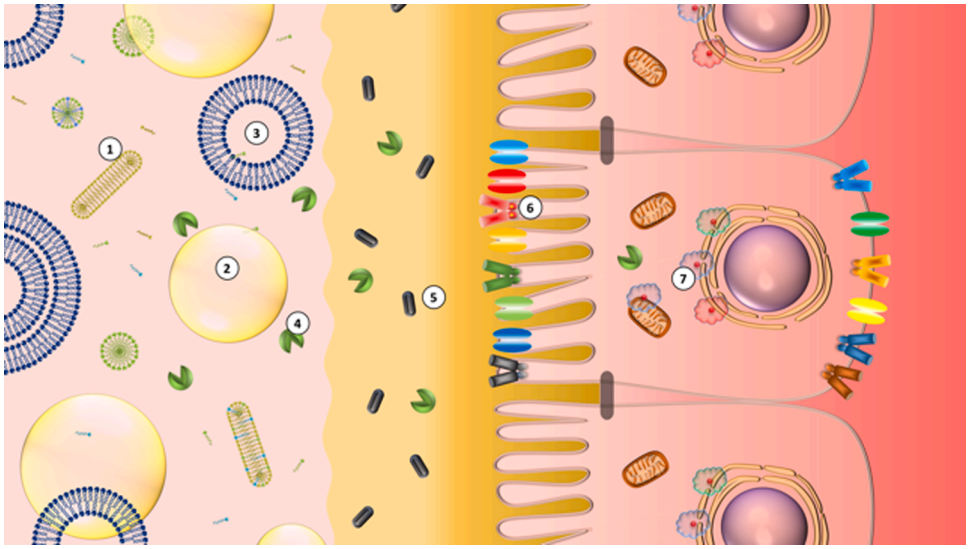

Fig. 6.2. Graphical representation of the intraluminal environment presenting the key factors that affect oral drug absorption processes (1) micelles, (2) lipid droplets, (3) lipid vesicles, (4) digestive enzymes, (5) mucus layer with bacteria, (6) drug transporters and (7) metabolizing enzymes.

duodenum (using a common naso-gastric tube) or the proximal jejunum (using a custom-made tube). The further the tube needs to pass along the small intestine, the longer it takes to manipulate and the more difficult sampling becomes. For this reason, the reports on collecting of fluids from the distal parts of the jejunum or from the ileum are rather scarce. In recent years, colonic fluids have been collected, either by means of a tube positioned during a colonoscopy or by direct aspiration through the colonoscope. Due to the limited fluid volume in the colon, sampling is much more challenging. Aspirated human fluids can be divided into blank fluids without any drug present, and fluids aspirated after intake of a drug product to investigate the luminal drug behaviour (Riethorst et al., 2016a). Fluids can be aspirated after a period of fasting or after intake of a meal, representing the fasted and fed state, respectively. Apart from characterizing aspirated fluids with respect to their composition and possibly drug concentration, these fluids can be used as the ultimate reference media in assays that evaluate absorption-related processes as depicted in Fig. 6.1.

The following paragraphs illustrate how this helps to shed light on the impact of the complex fluid composition on drug absorption. For a detailed description of the aspiration technique itself and its applications, we recommend a recent review (Augustijns et al., 2020)

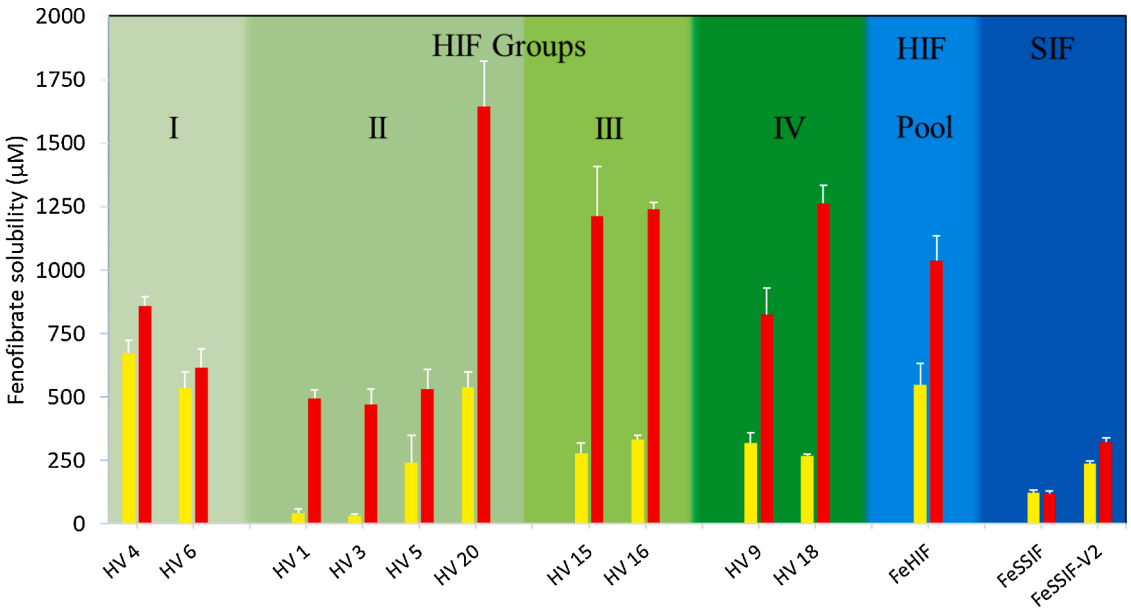

Fig. 6.3. Solubility of fenofibrate in the micellar layer (yellow) and in the combined micellar and lipid layers (red) of fed state human and simulated intestinal fluids (HIF and SIF). The green areas depict the solubility's in human fluids from different healthy volunteers (HV), grouped by their ultrastructure (I: micelles, II: micelles + vesicles, III: micelles + large vesicles + lipid droplets, IV: micelles + small vesicles + lipid droplets). The blue areas depict the solubility's in a pool of human fluids from all volunteers (FeHIF, light blue) and in simulated fluids (FeSSIF and FeSSIF-v2, dark blue). The results are expressed as mean + SD ($n = 3$). Figure adapted from Riethorst et al. (2016c). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

6.2.1. *Complex composition of human intestinal fluids*

Human intestinal fluids are a highly complex mixture of endogenous secretions and dietary products, constantly changing in both time and position along the intestinal tract as digestion and absorption proceeds. In Fig. 6.2, a schematic representation of the intraluminal environment is depicted illustrating the key actors in both drug dissolution and permeation. Lipid droplets and colloidal structures including (mixed-) micelles and vesicles (Fig. 6.2), create local hydrophobic environments within the gut in which lipophilic drugs can be solubilized, elevating their apparent solubility. These structures consist of endogenous bile salts and phospholipids, combined with dietary lipids and their digestion products. As mentioned, these structures are constantly changing due to enzymatic digestion and/or absorption of their constituents (Fig. 6.2). In addition to this enzymatic digestion, commensal bacteria located in the lumen of the colon and to a lesser extent the small intestine (Fig. 6.2) further metabolize dietary, endogenous and drug products (Enright et al., 2017). It is clear that a single formula of simulated intestinal fluids does not fully capture this complex and dynamic nature of human fluid composition, especially when combined with intra- and interindividual differences in secretions, diet and digestion. This challenges the accurate prediction of critical biopharmaceutical properties and processes, as becomes clear when using human intestinal fluids in, for instance, solubility and permeability assays.

6.2.2. *Human intestinal fluids in drug solubility, dissolution and supersaturation assays*

The complexity of human intestinal fluids highly increases upon intake of food, which mediates both secretion and digestion. This has important consequences for the solubilisation of lipophilic drugs that suffer from a poor aqueous solubility. In this respect, a relatively good correlation was obtained between the solubility of a series of poorly soluble protease inhibitors in simulated versus human fluids for fasted state conditions but not for fed state conditions (Wuyts et al., 2013). The challenges in accurately predicting postprandial intestinal drug solubility based on simulated fluids were confirmed by a meta-analysis of all published solubility data in human intestinal fluids (Augustijns et al., 2014). When visualizing the ultrastructure of intestinal fluids, Riethorst and colleagues. (Riethorst et al., 2016b) demonstrated that the diverse variety of structures present in postprandial human fluids, including large lipid droplets, vesicles, and micelles in different shapes and sizes, is only partially represented in the commercially available fed state simulated fluids FeSSIF and FeSSIF-v2, which focus on the micellar phase (Vertzoni et al., 2012). As a result, the solubilisation of lipophilic drugs by lipid droplets and large lipid vesicles, occurring in fed state human intestinal fluids, is not accurately simulated or predicted (Riethorst et al., 2018). In Fig. 6.3, this is illustrated for the drug fenofibrate, for which the solubility in postprandial conditions is greatly increased in the presence of lipid vesicles and droplets in most human intestinal fluids, but not in simulated fluids.

Besides affecting the apparent solubility of drugs, the complex composition of intestinal fluids may play a role in creating and maintaining a metastable, supersaturated state of poorly soluble drugs, in which the dissolved concentration of the drug (temporarily) exceeds its solubility. The extent and duration of supersaturation, which are critical for the impact on drug absorption, are dependant on the composition of the test media. Bevernage and co-workers (Bevernage et al., 2010, 2011) demonstrated that the stability of supersaturation in human intestinal fluids, both in the absence or presence of precipitation-inhibiting excipients, cannot always be predicted by using simulated fluids. Recent mechanistic studies, illustrating that different bile species and phospholipid degradation products may have a different effect on the stability of supersaturated drug solutions, support the added value of including human intestinal fluids as reference media in supersaturation and precipitation assays (Elkhabaz et al., 2019; Enright et al., 2017).

6.2.3. *Human intestinal fluids in permeation assays*

Following dissolution, a drug must permeate through the gut wall to enter the bloodstream. Traditionally, intestinal permeability assays are performed using simple buffers as the donor medium; however, the use of human intestinal fluids in these assays has revealed that their composition may affect drug permeation in more than one way.

First, intestinal fluid constituents may affect the activity of drug transporters and metabolic enzymes present in the enterocytes. For

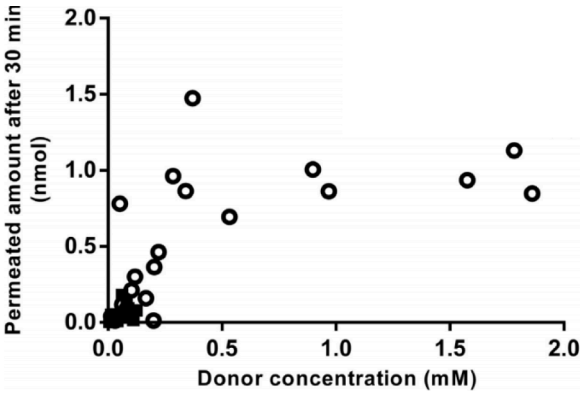

Fig. 6.4. Relationship between the amount of itraconazole permeating through Caco-2 cells (30 min) and the dissolved concentration of itraconazole in duodenal fluids aspirated after intake of a cyclodextrin-based oral solution. Closed squares and open circles represent samples from the condition with and without concomitant water intake, respectively (mean, $n = 2$). Figure from Berben et al. (2017).

instance, Deferme and colleagues demonstrated that the activity of the efflux carrier P-gp is attenuated in the presence of human intestinal fluids, due to an inhibiting effect of bile salts (Deferme et al., 2003). This was confirmed by Brouwers and colleagues, who assessed the permeation of amprenavir across Caco-2 cells using intestinal fluids aspirated from volunteers following intake of a solubilizing formulation of the drug (Brouwers et al., 2006). Although amprenavir is a P-gp substrate, it emerged that the combination of high, solubilized amprenavir concentrations and the presence of bile salts and the solubilizing excipient TPGS, both acting as P-gp inhibitors, abolished the P-gp effect. Such observations have stimulated the use of biorelevant conditions in not only solubility and dissolution assays but also in permeation assays.

When using simulated intestinal fluids in permeation assays, differences with human fluids should be considered when interpreting results. This was illustrated in a study that investigated the mechanism of amprenavir absorption starting from its prodrug fosamprenavir (Brouwers et al., 2007). In the presence of human intestinal fluids, dephosphorylation of fosamprenavir by phosphatases in the apical membrane of Caco-2 monolayers resulted in supersaturation and increased permeation of amprenavir. However, this effect could not be simulated by using standard fasted state simulated intestinal fluid (FaSSIF), as the enzymatic dephosphorylation of fosamprenavir appeared to be inhibited by the high and physiologically irrelevant inorganic phosphate concentration in FaSSIF.

In addition to direct effects of intestinal fluid constituents on the biochemical barrier, intestinal colloids may strongly affect the permeation of lipophilic compounds. As mentioned above, the apparent solubility of such compounds is typically increased by their interaction with micelles, vesicles and lipid droplets present in human intestinal fluids. However, this solubilisation does not necessarily imply an increased permeation, as the solubilized fraction of a compound may be entrapped in the colloidal structures and not readily available to permeate (in contrast to the free fraction in the aqueous phase). This is illustrated by experiments reported by Hens and colleagues (Hens et al., 2015) who evaluated the permeation of fenofibrate from human intestinal fluid samples, aspirated after intake of micro- and nanosized fenofibrate in fasted and fed state conditions. Even though a substantially higher fenofibrate concentration was measured in fed state fluids, no increased permeation across an artificial membrane was observed. This explained the absence of a positive food effect on the absorption of fenofibrate, despite strong solubilisation. In addition, the study revealed that the use of a commercially available fed state simulated medium (FeSSIF-v2) could not fully predict the observed food effect with human fluids, due to inadequate simulation of solubilisation and entrapment. The effect of colloidal entrapment in postprandial human intestinal fluids on the permeation of lipophilic compounds was also seen in the Ussing Chambers apparatus with excised rat tissue (Wuyts et al., 2015), and in the intestinal *in situ* perfusion model in rats (Stappaerts et al., 2014).

The interplay between solubilisation and permeation is not only relevant to food effects but also to solubilizing formulation excipients in intestinal fluids. Berben and colleagues (Berben et al., 2017) studied the impact of intraluminal dilution on the intestinal absorption of itraconazole after intake of a cyclodextrin-containing oral solution. Without additional water intake, intraluminal dilution of the cyclodextrins was reduced and stronger complexes were formed with the lipophilic drug itraconazole. The resulting increase in itraconazole solubilisation in the intestinal fluids, however, did not lead to improved absorption. When the intestinal fluid samples were applied onto a Caco-2 monolayer, it became clear that the solubilized itraconazole was trapped in the strong cyclodextrin complexes and not resulting an increased permeation. This is depicted in Fig. 6.4 where the permeated amount of itraconazole across the Caco-2 monolayers reaches a plateau despite increasing concentrations in solution.

All these observations on drug permeation from human intestinal fluids point out the importance of carefully balancing intestinal solubilisation and permeation when evaluating the absorption of lipophilic compounds.

6.3. *Conclusion*

The examples mentioned above demonstrate the benefit of using human intestinal fluids as the ultimate reference medium to unravel the complex impact of the intraluminal environment on absorption-related processes. It is important to note that the use of aspirated human fluids is by no means an effortless undertaking. First, it is clear that the invasive aspiration protocol, involving intubation with GI catheters, limits the availability of human intestinal fluids, as it requires specialized equipment (e.g., fluoroscopy to guide the positioning of the catheters), training and approval of an ethical committee. In addition, the stability of intestinal fluids is limited due to the volatile bicarbonate buffer (especially in fasted state fluids), the presence of digestive enzymes, and bacteria. While most factors can be stabilized by adding inhibiting agents (e.g., protease inhibitors, lipase inhibitors) and by freezing, the instability of the buffer during freeze-thaw cycles, centrifugation and handling of the samples, make it hard to adequately capture the role of pH (Litou et al., 2020). Because of these availability and stability issues, human intestinal fluids will never be used on a large scale during drug development. The results obtained with these fluids should therefore always be considered as a stimulus and guidance to develop and optimize simulated fluids with improved biorelevance. In this respect, current efforts aim to improve the simulation of intestinal solubilisation of lipophilic drugs and its interplay with permeation.

Urged by experiences in clinical practice, increased attention is currently being paid to the development of robust oral drug products that reduce the risk on unwanted variations in the rate or extent of drug absorption. Recent studies by Riethorst and colleagues have demonstrated a large interindividual variability in both composition and ultrastructure of human intestinal fluids, even when aspirated from healthy adults under standardized conditions (Riethorst et al., 2016a; Riethorst et al., 2016b). As such variability is not implemented in the currently available simulated intestinal fluids, human fluids aspirated from different individuals may be used to better understand the sensitivity of drug behaviour and absorption to variations in fluid composition and ultrastructure. In this respect, it has been shown that the postprandial solubilisation of lipophilic drugs is more pronounced for subjects with lipid vesicle-rich intestinal fluids as compared to subjects whose fluids are lacking these structures (Riethorst et al., 2018).

Exploring the effects of interindividual variations in intestinal fluids becomes even more relevant in the framework of the current paradigm

shift in absorption-related studies from the general (healthy) population to specific age- or disease-related populations in which the heterogeneity is further increased. Unfortunately, invasive testing in these specific populations poses ethical restraints, especially as there is no direct benefit for the patient. By collecting fluids during scheduled procedures (e.g. abdominal surgery, naso-gastric feeding, and endoscopic procedures) these restraints might be overcome, as was recently illustrated with the collection and characterization of gastric and intestinal fluids from the paediatric population (Pawar et al., 2021; Van Den Abeele et al., 2018).

Overall, multiple opportunities exist for future use of human intestinal fluids in absorption-related assays. The outcome of such studies will provide critical reference data to optimize simulation media and predictive models that allow mapping the sensitivity of drug candidates to the dynamic and highly variable intestinal environment, thereby contributing to the development of effective and robust oral drug products.

7. Microdosing

Clive G. Wilson, Strathclyde Institute of Pharmacy & Biomedical Sciences, Glasgow, U.K.

- The introduction of intravenous microdosing, using >500 nCi amounts of [^{14}C]-labelled drug allowed the exploration of the disposition pathways of the compound and metabolites after single doses. The development of high sensitivity mass spectrometers gradually widened applications to include non-labelled substrates.
- Extrapolation of data from microdosing to the clinical scenario has limitations when non-linear behaviour is encountered, for example when a pathway is saturable.
- Microdosing has good utility in paediatric medicine development and in the patient with chronic disease, especially if the oral dosing can be added within a routine monitoring or treatment of a patient.
- Innovation in microdosing has been consistent in oncology, where systemic administration of large molecules is employed. Newer applications for antibody distribution studies have introduced SPECT labels to overcome the limitation of fast decaying radionuclides for compounds with longer distribution phases. Target-specific evaluations have also received attention

Summary

Microdosing is an extremely useful probing tool, using subtherapeutic concentrations of drugs to investigate the disposition pathway. Introduced in the early 2000′s using (^{14}C)-labelled compounds, improvement in analytical technology including advanced mass spectrometers and liquid chromatography/tandem mass spectrometry facilitated the applications, moving from radio-isotope based measurements, through stable isotopes to native drugs. The use of mixtures of drugs (cassette dosing) to simultaneously probe key metabolic and efflux pathways after oral absorption may be problematic however, due to absorption non-linearity associated with efflux or CYP isoform interactions. The applications to paediatric dosing and to cancer theranostics are considered together with more general connections to physiologically-based modelling.

7.1. Introduction

At an early stage of development, usually between the preclinical and early clinical evaluation, there is an appetite to collect data on deposition and clearance of the drug under investigation. Carbon-14 labelled drug studies in animals to determine organ deposition and clearance were previously well established but accelerated mass spectroscopy allowed a combination study of an intravenous dose with a carbon-14 tracer at low activity (100 - 500 nCi) to be simultaneously administered with an oral dose to man (Lappin et al., 2013). This procedure was not true microdosing since the full dose was administered orally but the protocol potentially sped up the decision process for large pharmaceutical companies. At the beginnings of drug development, relatively small amounts of the drug are available, and the safety of the compound and persistence and identity of metabolites would not have been fully evaluated so at least in theory, the technology fits the need. It is true that fewer drugs now fail in development due to pharmacokinetic considerations, so such investigations are not necessarily mandatory. Nevertheless, the use of isotope-labelled formulations at sub-therapeutic doses assists in establishing key human pharmacokinetic parameters, which can be linked to pharmacodynamic data. Regulatory guidelines on the use of single microdose studies were published in the early 2000′s as reviewed by Maeda and Sugiyama (Maeda and Sugiyama, 2011). The dose is administered at 1/100th of the pharmacological dose as a maximum of no more than 100 μg of the drug and as such, solubility issues are not generally encountered. This has been termed the Phase 0 approach as advocated by early proponents of the microdosing technique (Burt et al., 2020). The drugs are given at the No Adverse Effect Level (NOEL) and become especially useful for potential APIs selected for cancer treatment where there is likely to be a lower safety margin. The techniques used were originally based on a nuclide tag of an unstable nuclide, typically ^{14}C-labelled drugs and detection by Accelerator Mass Spectrometry (AMS), by measuring the ratio of ^{14}C/^{12}C ratio.

7.2. Microdosing

7.2.1. Use of stable isotopes

The introduction of stable isotopes in microdosing studies was obviously attractive as it avoided the necessity of synthesising the radioactive metabolites alongside the drug and permitted other designs of safety trial. As deuterated compounds are widely available as LCMS probes, deuterium/hydrogen ratios were another obvious step, but it was found that the carbon-deuterium bond in the drug analogue resulted in an alteration of metabolic rates in the drug degradative pathway. This is known as the kinetic isotope effect and is usually evaluated ahead of the clinical trial (Roosendaal et al., 2020a). Roosendaal and colleagues have described the use of “micro dosed” i.v. deuterium labelled imatinib to determine absolute bioavailability of the drug which was given intravenously to patients with a history of GI tumours (Roosendaal et al., 2020b). The deuterated drug was given alongside administration of oral and i.v. doses. A strong reduction in expected bioavailability was observed, perhaps due to regular concomitant ingestion with food although previous studies have noted that long term treatment with imatinib (<90 days) is associated with a decrease in absorption of around 30% (Eechoute et al., 2012). Overall, the use of i.v. microdosing coupled with oral dosing studies significantly reduced the number of studies needed to establish the true PK time profile.

7.2.2. No label, cassette microdosing

Developments in liquid chromatography /tandem mass spectroscopy (LC/MS/MS) facilitated the detection at the pg/mL level and the introduction of non-label studies. In addition, Maeda and Sugiyama (2011) addressed the advantages of cassette dosing to facilitate a more rapid determination of possible drug interactions following oral dosing, as long washout periods would be avoided. Ieiri and colleagues (2012) described the comparison within a cassette microdose of celiprolol, fenoxafenadine and atenolol. The drugs were given in an oral cassette microdose then a therapeutic dose, with and without grapefruit juice in volunteers who were genotype matched for the SLCO2B1 gene. The SLCOB1 gene codes for the organic ion transporter polypeptide, which is the predominant transporter involved in the celiprolol-naringin intestinal absorption interaction. Polymorphism should therefore exert a strong influence on AUC. For celiprolol, the dose-normalised reduction in AUC by grapefruit juice was much more evident for the microdose than for the therapeutic dose (Ieiri et al., 2012). This example illustrates a general problem associated with microdosing, which can occur when

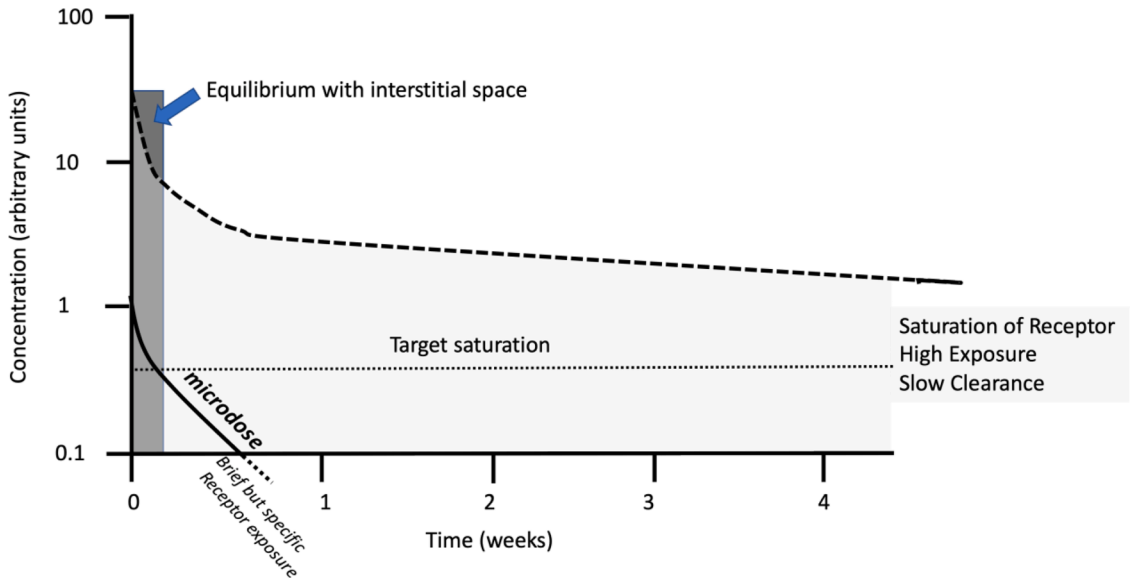

Fig. 7.1. Comparison of the expected kinetics of an antibody given at microdose and therapeutic dose amounts. Adapted and redrawn from Bergstrom, 2017.

the relationship between dose administered, and the amount of drug absorbed, deviate from linearity. The absorption process is a mix of non-saturable and the saturable components, so the latter will result in differences between the clinical and the microdose level. Food will also affect the environment for the presentation of the drug.

Frick and colleagues had originally noted that there are analytical problems associated with poor solubility and with isobaric fragments. Compound analogues generate similar daughter ions and therefore very similar compounds in a cassette selection might not be distinguishable (Frick et al., 1998). The avoidance of uncertainty might be lessened by selecting compounds in such a series, which differ by 16 mass units, equivalent to an oxidative step (Chavez-Eng et al., 2018). Maeda and Sugiyama (2011) avoid the issues of solubility encountered earlier by Frick and colleagues because of the low doses used and the pharmacological interactions that bedevilled the early studies are mitigated (White and Manitpisitkul, 2001).

There are many interesting applications of micro-dosing cocktails, essentially cassette dosing, to dissect out the impact of specific transporters in drug-drug interaction studies as used by Chavez -Eng and colleagues for statins and metabolites (Chavez-Eng et al., 2018). The group used a cassette of containing pitavastatin, pitavastatin lactone, rosuvastatin, atorvastatin, 2-OH atorvastatin, and 4-OH atorvastatin). The use of the metabolites facilitated the optimisation of the analytical techniques to minimise of interconversion of the compounds during processing prior to injection on the column. This illustrates an expected stability problem in the handling of such small amounts of drug. Midazolam was used as a classical probe for CYP3A expression and dabigatran as a marker for p-glycoprotein efflux.

7.2.3. The advantages of infusion versus bolus

Barbour and Fossler published evidence from two examples that one hour infusions gave better discrimination of the pharmacokinetic parameters associated with two experimental drugs compared to kinetics determined using a two-minute i.v. bolus (Barbour and Fossler, 2018). The issue is particularly important when the drug has a rapid distribution phase, which is perhaps not surprising as circulating blood time is around 20 s. The circulation time appears to increase linearly over a weight range of 3 to 60 kgm, infant to young adult (Seckei, 1936).

Bergstrom reviews the applications of PET tracers in the development of protein therapeutics and discusses many of the inherent problems in radionuclide imaging. Imaging, as will be discussed, adds much to the knowledge of organ distribution although the species accumulating - drug or metabolite - might not be distinguishable. Dynamic sequences, with blood sampling during the acquisition may assist interpretation (Bergstrom, 2017). The author mentions that metabolic PET tracers are too short lived and classical SPECT labels including ^{111}In, ^{124}I and ^{89}Zr with half-lives of a few days are more useful, particular to map the distribution of antibodies.

7.2.4. Target mediated disposition

Large molecular constructs such as antibodies are not absorbed orally, and therefore are justifiably beyond the normal scope of consideration in this review of GI absorption. They are mentioned because of another important pharmacokinetic phenomenon: target mediated deposition. In the gut, difficulties in data interpretation can arise from non-saturation of efflux and metabolic pathways and within the systemic circulation, slow equilibration processes can prove problematic. Many anti-VEGF agents such as bevacizumab bind to external receptors and are slowly internalised as they pass through the endothelium. This is nicely illustrated in a classic paper examining the distribution of bevacizumab following intravitreal injection into monkeys in which the antibody revealed by immunostaining accumulated at the inner limiting membrane of the vitreous humour before entering the deeper retina (Heiduschka et al., 2007). A proportion of antibody dosed will pass through by Fc coupling - the linking of the Fc fragment of the antibody to specific membrane bound receptors on mast cells and lymphocytes associated with the tissue surface. Within the cell, internalisation of the antibody-Fc complex leads to various fates, including degradation and shuttling of the receptor back to the surface. Iodine labels are facile to degrade, leading to a rapid loss of the tag from the cell whereas residual radionuclides remain. A very small dose of a labelled large biological, such as in a microdose, will be removed by liver and spleen and of course Fc- receptor interaction. The clearance of a labelled antibody following microdose or therapeutic dose is illustrated in Fig. 7.1. The microdose typically shows a brief but targeted exposure whereas circulating levels of the antibody at therapeutic doses persist, often with large amounts of uptake by the Kupfer cells of the liver, which may obscure identification of tumour sites in the abdomen. Using careful timing and control of dose, since equilibrium states are achieved slowly with large molecules, the optimum concentration used as a microdose can be determined and patient heterogeneity explored. This assists on stratifying patients to maximise treatment efficacy. Kunos and colleagues comment that microdosing studies in oncology are very valuable, informing the team about patient selection and biopsy strategies

(Kunos et al., 2021).

Target-mediated disposition is the goal in cancer treatment and many studies compare results in rodents with those in patients. This elucidates both the suitability of the animal model and the extrapolation across species. Moon and colleagues described the tissue kinetics of (^{18}F) paclitaxel in a novel oral lipid composition using a microdosing PET based study in tumour-bearing mice and two patients with metastatic breast cancer (Moon et al., 2021). The formulation achieved peak concentrations in the target breast tissue around 5–6 h after dosing. The intention is essentially to achieve a measurement that is a step beyond AUC - i.e., to relate that proportion of the dose in the post absorption phase that distributes to the region of therapeutic interest and look to see how it is managed by the oral formulation.

7.2.5. Microdosing and regional drug absorption

Utilising microdosing studies for oral drug absorption in the gut is generally hampered by saturable processes as has been described; however, Grass and colleagues describe an interesting application to study buccal absorption of midazolam (Grass et al., 2021). The experiment attempted to explore whether buccal absorption of midazolam, a probe drug for intestinal CYP3A activity, might influence the data obtained from midazolam microdosing solutions. The investigation showed that buccal absorption within 16 s would be sufficient to influence the outcome of a CYP3A screen indicating that holding the formulation in the mouth before swallowing would be likely to be a confounding variable in microdosing trials.

7.2.6. Intra-targeted microdosing

The concept of intra-target mediated microdosing was crystallised in a suggestion by Burt and co-workers (Burt et al., 2017). They proposed a design of microdosing based on close arterial injection of a microdose to the tissue of interest. By pulsing the dose to an organ by control of arterial supply, pharmacologically relevant concentrations would be briefly generated before rapid dilution in the remaining circulation. The objective was to detect the generation of relevant biomarkers and perhaps generate preliminary pharmacokinetic data. There were no suggested examples for the use of this tool to study drug absorption in the gut.

7.2.7. Microdosing, pop-PK and renal disease

An important application of microdosing is the investigation of key groups in the population, including children and patients with chronic disease. These are identified gaps in models of population PK. The avoidance of pharmacological effects is important, almost the opposite of the criteria sought in intra-target microdosing. The formulation of paediatric medicines needs to advance whilst additional intervention remains managed. Mooij and colleagues described the use of an oral microdose of (^{14}C) paracetamol, which was given together with an intravenous therapeutic dose of the same drug (Mooij et al., 2014). The purpose of the study was to take an opportunity to gather data during ongoing patient management to support POP PK and physiologically based pharmacokinetic models in a vulnerable population where drugs are given at a specific age for the first time. The authors comment that the extremely low radiation dose associated with the procedure was not of concern to the parents and the advantage of a more sensitive procedure protects the young patients from pharmacological effects of larger doses then needed for stable isotope microdosing.

Renal disease exerts a wide effect on the pharmacokinetics of many drugs, including those whose primary elimination pathway is not through urine. Tatosian and colleagues investigated cohorts of healthy controls against patient groups with mild, moderate, and severe renal impairment with regard to intestinal transporter activity (Tatosian et al., 2021). The microdosing cassette included midazolam (an inhibitor of CYP3A4), dabigatran etexilate (an inhibitor of P-gp), pivavastatin (an OATP1B substrate), rosuvastatin (transported by OATP1B and BCRP) and atorvastatin (disposition dependant of CYP3A4, OATP1B, BCRP, and P-gp activity). The oral cocktail was administered with and without rifampicin to each of the treatment populations. There was no effect of rifampicin on midazolam kinetics, but progressive levels of renal impairment decreased the activity of efflux transporters increasing the AUC for pivavastatin, rosuvastatin and atorvastatin. The effects on classical endogenous markers of OAT1B (bilirubin, sulphated bile salts and coproporphyrin I/III) were minimal, with levels similar to those seen in the healthy volunteers. This points to an involvement of mechanisms other than OATP1B being regulated by renal disease.

7.2.8. Standardising microdosing cocktail mixtures

It becomes apparent that within a cassette dose, the opportunity to explore all the players of interest is available including CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Adoption of a mixture of drugs, which preferably are all single transporter substrates, has been explored although as Tornio and colleagues observe, some of the index compounds are no longer commercially available in certain countries (Tornio et al., 2019). These authors describe the selection of components into formulations such as the 'Basel cocktail' and the 'Geneva cocktail'(which also includes fexofenadine as an index drug for p-glycoprotein) in the validation of drug-drug interactions.

7.2.9. Microdosing in a non-analytical context

Finally, dosing very small amounts of potent drugs can result in profound pharmacological effects. Authors often refer to this as 'microdosing' although the intention is clearly different to metabolic profiling. For example, Pasquale and colleagues report on the use of piezo printing to deliver latanoprost at volumes of 8 uL containing 0.4 μg of the drug (Pasquale et al., 2018). The mean reduction in intra-ocular pressure was 25 to 30% on day 2. Similarly, Sandhu and colleagues refer to microdosing when giving small doses of opiate during drug transition in pain management, the intent being to manage withdrawal symptoms (Sandhu et al., 2019).

7.3. Conclusions

Burt and colleagues observed that although the FDA identified microdosing as a key innovative approach in several documents from 2006 through to 2012, the industry has been slow to embrace the microdosing approach (Burt et al., 2020). In a study of an abandoned candidate drug for treatment of malaria, Okour and colleagues reported the use of microdosing to evaluate a candidate drug by intravenous dosing where conventional PK analysis gave widely divergent results. Based on the intravenous data, the characteristics were clearly evaluated, and the clearance of the drug judged too fast to provide a useful edge over current front-runners. Oral microdosing was then shelved (Okour et al., 2018). It is apparent that intravenous microdosing is more widely used than oral microdosing, although there is a constant trickle of reports where oral microdosing is pursued provided saturation kinetics are appropriately assessed. A further issue is that over time, a drug may show time-dependant kinetics, which may escape notice in probing drug-drug interactions and attention to the interval between dosing 'perpetrator" and 'victim" is also necessary as noted in the Tornio review. These authors suggest PBPK modelling provides an appropriate tool to explore interpretation of the data.

From an examination of the reports in the literature, it seems that the trends in microdosing will diverge for oral absorption of small molecules and systemic administration of large constructs. The use of microdosing in nuclear medicine proceeds and guides theranostics where the use of an energetic radionuclide emitting beta radiation can be justified by target assessment using a microdose of a gamma emitting analogue, typically a monoclonal antibody. In contrast, in oral medicine the use of microdosing will generally be restricted to smaller lipophiles, which could be preferably measured in a biological matrix as stable labels.

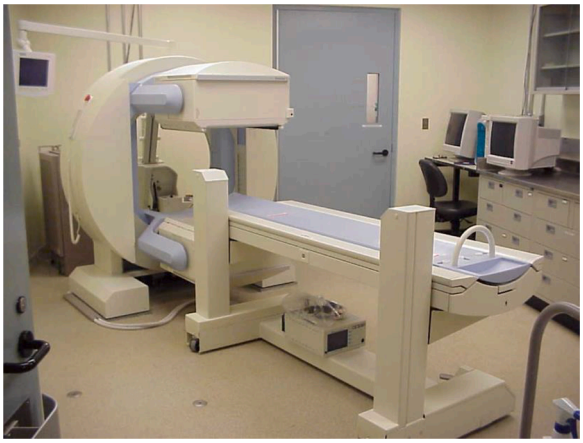

Fig. 8.1. Typical modern gamma camera with gantry.

8. The study of drug absorption using nuclear medicine

By Clive G. Wilson, Strathclyde University, Glasgow, U.K, and J. Arturo García-Horsman, University of Helsinki, Finland.

- Nuclear imaging is a well-established non-invasive technique that reports the quantitative distribution of gamma labelled radiopharmaceuticals in real time, in a safe and sensitive way.
- Scintigraphy is widely applicable to the study of GI motility and been used to observe food and formulation movement throughout the GI tract.
- Scintigraphic and tomographic methods offer more convenient strategies when the data are anatomically contextualised.
- Special attention should be taken to understand the tracer behaviour in order to validate the observations following administration of a matrix labelled with a radionuclide.
- Modern multimodal SPECT or PET along with CT, MRI or optical imaging, offer robust methodologies to follow drug and drug delivery system longitudinal dynamics, kinetics and disposition *in vivo*.

Summary

Despite its poor anatomical contextualisation, planar gamma scintigraphy has been a benchmark quantitative technique to follow dosage behaviour along the GI track *in vivo*, allowing the position of the dosage form to be related to the systemic concentrations measured from the blood matrix. One of the key issues of nuclear imaging is the selection of the molecular tracer and ideally, it is desirable that one of the atoms of the compound under investigation can be substituted by a suitable radioisotope. The nuclide selected should have good positron emission tomography (PET) or single photon emission computed tomography (SPECT) attributes. This is rarely the case, and modifications to the parent structure or matrix are made to attach a gamma-emitting radionuclide label. Inevitably, this changes the physicochemical properties of the labelled probe, compared with the parent molecule. In all cases, the stability of the compound in the GI environment, or if desired once it has entered the circulation, must be determined as a primary step to allow data interpretation. Many commonly used radiopharmaceuticals have physically short half-lives, which limit the length of the investigation. The development of SPECT, where longer-lived isotopes could be employed, allowed distribution and slow equilibration phases to be followed for longer times than with PET. Nuclear imaging has proved applicable when labelling large structures including proteins liposomes and nanoparticulates when the relative size of the radiolabel becomes less relevant. The possibility to introduce labels in different parts of a drug delivery system provides a valuable increase in the knowledge gained in each experimental procedure. For GI investigations, the target specificity of the delivery system, the exposure and the dispersion of the different components can be routinely determined by nuclear imaging.

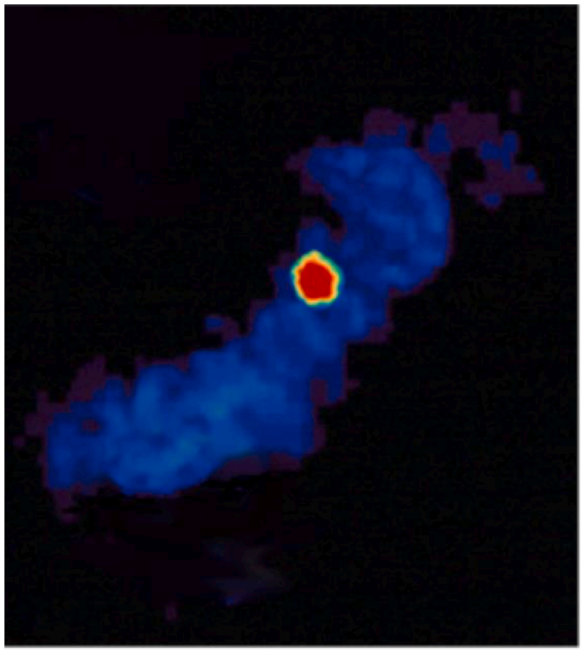

Fig. 8.2. Using a drink of (^{99m}Tc)-labelled Liquid (blue) in a volunteer to outline the stomach to determine the position of a capsule labelled with (^{111}In)-labelled amberlite resin (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In this paper, we review the development of nuclear imaging technology on the drug absorption research, focusing on the advantages and pitfalls of various techniques and highlight few examples, where nuclear imaging has given insights on the behaviour of oral formulations. Innovation often occurs in fields which are parallel to a particular interest but it important that the researcher is aware of innovative advances and for this reason, we will refer to some non-oral applications within this article

8.1. Introduction

The advent of the Anger gamma camera, single positron emission tomography (SPECT) and the wider availability of radiopharmaceuticals based on technetium-99 m and other short-lived gamma emitting isotopes drove the science of nuclear medicine. By 1980, a wide range of radiopharmaceuticals were described (Hardy and Wilson, 1981), gamma cameras became available for research and a new era formulation development began. A gamma camera and gantry are illustrated in Fig. 8.1. As can be seen, the machine is large and heavy because of the shielding necessary to provide undisturbed, collimated photons into the detector.

Nuclear imaging is mainly used as diagnostic tool, or to measure the prognosis of radiotherapy. The use has increased tremendously in the last few decades applied to formulation development and pharmacokinetics. Appropriate new molecular entities, with the substitution of an atom with an imaging radioactive isotope, have been used to measure bio-distribution and kinetics, evaluate off target exposure and ADME properties, at preclinical stages of compound or formulation selection. The use of nuclear imaging at later clinical stages of drug development has been under-represented, especially in formulation development of oral drugs, which constitute most developed drug formulations.

8.2. Nuclear imaging and drug absorption

8.2.1. Scintigraphy

Scintigraphy is considered as a benchmark for tracking the behaviour

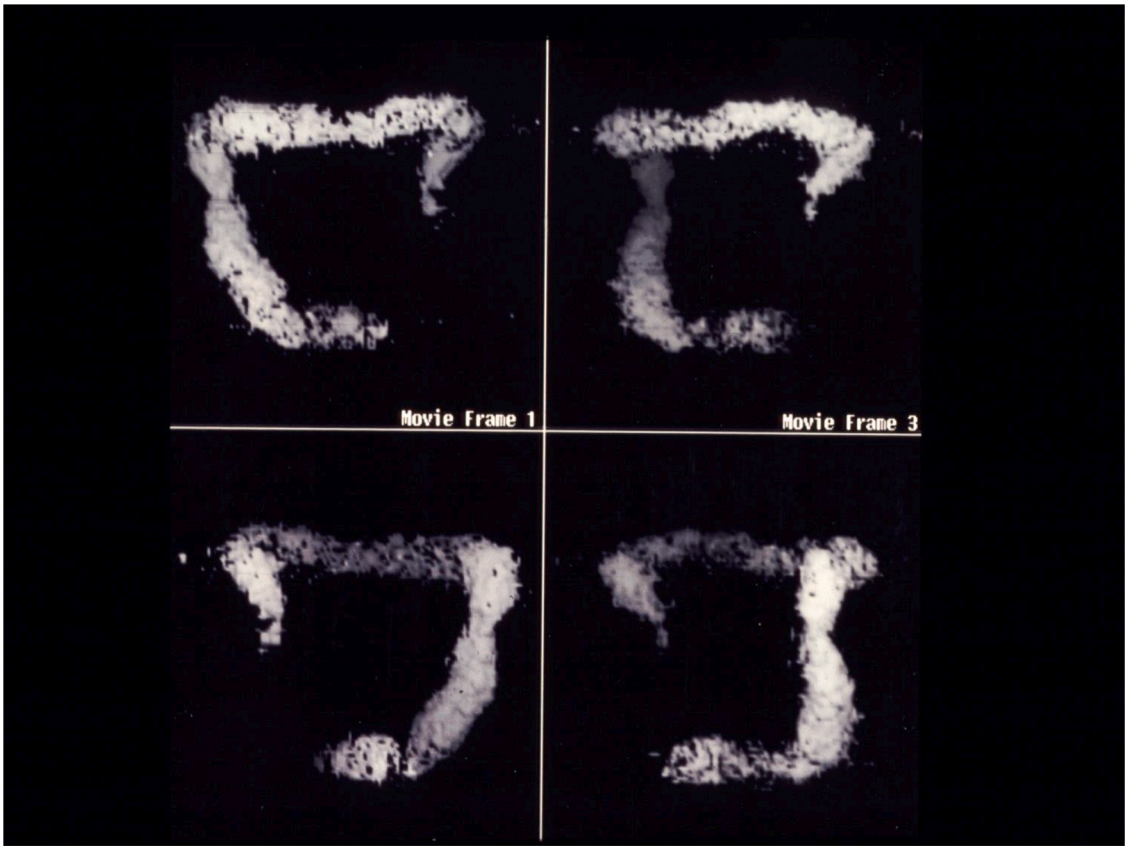

Fig. 8.3. Three dimensional images of abdomen and pelvis of (111In) delivered from a time pulsed system (Pulsincap, 5 h release time).

of a dosage form along the GI tract (Schneider et al., 2019). It has good temporal and spatial resolution, making it a robust technique. Bennet and colleagues underlined the value of scintigraphy in determining the performance of a formulation, labelled with (^{113m}In)-aluminium hydroxide, measuring the gastric residence of an antacid formulation (Bennett et al., 1984). Simple chemical procedures in the radiopharmacy in this case proved extremely useful for in vivo evaluation of locally acting medicines in a manner that complemented pH monitoring.

A major disadvantage of scintigraphy is the lack of good anatomical information. In oral studies, the labelled tablet matrix often appears as a 'comet', with the principal disintegrating or dissolving object noted at the front of the propelled mass. To overcome the problem of identifying the regional location within the gut, operators use acetate screen overlays to track the distribution of radioactivity referenced to weakly-labelled external markers taped on the skin allowing allows serial registration of images. Other technical improvements to identify key anatomical landmarks (stomach and colon), include the use of simultaneous administration of a different label in food or a drink improving spatial reference (Van Gansbeke et al., 1991). In the example shown in Fig. 8.2, an orally administered capsule had adhered to the stomach wall on the inferior curvature, revealed by swallowing a labelled drink.

8.2.2. *Tomographic nuclear imaging*

In the past, scintigraphy techniques were far from convenient and versatile. The addition of anatomical screening, CT, or MRI, to tomographic nuclear imaging raised the cost of the study but the information obtainable from each scan increased tremendously and nuclear medical applications still have more room for exploitation. Processing of SPECT data allows 3-D rendering, pixels to voxels, which can be used in studies of internal gut wall disposition. A clear picture of mucosal coating was obtained with (^{111}In)-labelled Amberlite resin administered using a timed-release system designed for targeted drug delivery as shown in Fig. 8.3 (Perkins et al., 1995; Wilson et al., 1997).

SPECT is much cheaper than PET as a cyclotron is not needed to produce the target radionuclide. The most used SPECT tracers ^{67}Ga, ^{67}Cu, ^{111}I, ^{123}In, ^{153}Sm, ^{159}Gd, ^{166}Ho and ^{177}Lu are long-lived, allowing time for processing and recovery of high purity sources. In PET, gamma rays, formed when an emitted positron annihilates an electron, are emitted in approximately opposite directions allowing simultaneous detection of a single event by opposed detectors. This is not a feature of SPECT, which follows a single emission event and consequently the sensitivity can be ten times lower than PET (Lu and Yuan, 2015)

8.2.3. *Radionuclides and radiopharmaceuticals*

In the radiopharmacy, the simple salt (^{99m}Tc) sodium pertechnetate is often regarded as the grandfather radiopharmaceutical of nuclear medicine. The radiopharmaceutical can be prepared on site, eluted from a (^{99}Mo) generator, sometimes termed a moly or technetium cow. The physical half-life of technetium-99 m of 6 h, an energy of 140 keV and metastable decay which generates gamma rays without accompanying hard beta radiation lent itself well to clinical investigation. Over more than 25 years, nuclear medicine protocols to study GI tract function and dysfunction have evolved. These include sialoscintigraphy, used to examine the function of the submandibular gland after lithectomy (Nishi et al., 1987), methods usually based on technetium-99 m and indium-111 to follow oesophageal transit, gastric emptying, small bowel transit, and colonic transit.

The use of nuclear imaging for the study of drug delivery systems, including nano-pharmaceutical constructs, achieved a high profile as engineered selectivity flourished. The status of current medical materials is reviewed by the World Nuclear Association (https://www.world-nuclear.org/information-library/non-power-nuclear-applications/radioisotopes-research/radioisotopes-in-medicine.aspx) with a detailed technical account by Willowson, 2019. With more complicated

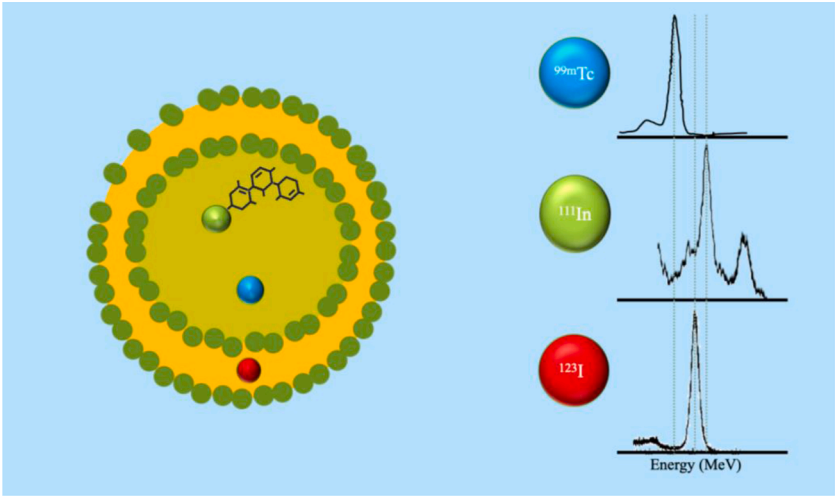

Fig. 8.4. A liposomal drug delivery system can be labelled at different positions. At the core of the vesicle, free (^{99m}Tc)-technetium radiopharmaceutical (blue ball) and a potential drug labelled with (^{111}In)-indium (green ball). The envelope is labelled with (^{123}I)-iodine (red ball). The energy peaks of each gamma emitting isotope can be resolved (left) and individual distributions detected by SPECT imaging. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pharmaceutical constructs including liposomes, a range of possibilities can be explored including surface labelling using radiometals and chelating agents. These steps can be completed post-assembly, whilst interior labelling is carried out during manufacture (Man et al., 2019).

The radionuclides suitable for gamma imaging, particularly metastable isotopes, do not occur in most drugs. PET overcame these limitations since molecules of interest can be labelled with extremely short-lived positron emitters including ^{11}C, ^{13}N and ^{15}O. Of the PET materials, (^{18}F) as (^{18}F)-fluoro-deoxy glucose is one of the most studied materials in early-stage monitoring as it has a half-life of 110 min. This compound has found applications in brain-directed protocols to search for metastatic disease.

8.2.4. *Safety*

The key feature of nuclear imaging is the non-invasive nature with the ability to quantify the presence, distribution, uptake, and clearance of a labelled compound / substrate of interest deep within the body. Thus, the clinician was able to measure the function of the GI tract, with guided biopsy collection and less reliance on the invasive tube endoscopy. Volunteer studies can be performed within exposures well within agreed limits. It should be appreciated that location and profession influence the background exposure. In Finland the average person's dose is 3.2 mSv per year compared to London at 2.7 mSv. Working as aircrew for a year adds an extra 2 mSv and volunteer studies typically receive 0.6 to 1.1 mSv per complete protocol, well within the recommended maximums. Nevertheless, we should be cautious. Radiation exposure and radioactive substances causes concern, not only in the lay population, but also amongst specialised professionals and is therefore tightly regulated.

The sensitivity of the modern gamma radiation detectors allows reduction in operator/subject exposure, even compared with a dental x-ray-based examination. Pico- or even femtomolar quantities of radiopharmaceuticals are employed at sub-pharmacological concentrations but permit accurate determinations of bio-distribution and elimination kinetics. Accordingly, scintigraphy and tomographic studies (PET or SPECT), are now more desirable than ever.

8.2.5. *More information per experiment*

Scintigraphic studies are free of the many artefacts associated with invasive sampling, with real-time data and a longitudinal follow up. Each subject in such a design can be its own control, increasing the statistical power of the study. The use of animal models has been less due in part to the differences between the gastric specific biochemistry and physiology of small animals (rodents) and humans. Despite this, assays in animals can be used to draw general outlines in the investigation of GI absorption properties of potential drug formulations. Animals such as pigs, which are closer in size and anatomy to humans, could be used in follow up studies (Zhang et al., 2013; Gonzalez et al., 2015; Henze et al., 2019).

8.2.6. *Use of nuclear medicine in drug permeation and distribution studies*

Nuclear imaging methods have been developed to measure small and large bowel permeability and more powerfully, a non-absorbed tracer can be combined with permeability markers. Non-scanning radionuclides, particularly (^{14}C) labelled compounds are used to determine GI tract injury or increased permeability in several diseases (Allen and Tulchinsky, 2013). A derivation, *microdosing*, is discussed earlier in the review. Some methods are based on the appearance of radioactively labelled sugars in serum or urine, after oral administration (Anderson et al., 2004). After absorption, these sugars appear in the bloodstream, where they are no longer metabolised and the urine, with distinctive kinetics depending on the site of absorption. Thus, some sugars are absorbed soon after leaving the stomach by active transport (glucose, galactose, fructose), others passively with various extents of bioavailability (xylose, mannitol, rhamnose), but others pass unchanged through the whole bowel and found unchanged in faeces (lactulose).

Sucrose is degraded after leaving the stomach, so sucrose detected in the urine reflects gastric permeability. On the other hand, non-metabolisable sucralose is absorbed through the small and large intestine. Therefore, urinary sucrose, lactulose/mannitol ratio, and sucralose levels reflect gastroduodenal, small intestinal and total gut (small bowel and large bowel) permeability, respectively (Menard et al., 2010; Farhadi et al., 2006).

As mentioned earlier, if a radioactive labelled drug can be orally administrated along with any of those 'standard' sugars and the excretion kinetics compared, clues on the absorption properties of the drug can then be drawn. As the kinetics of excretion are related to disruption of the permeability of the gut in disease, the assays can determine changes in absorption for pharmacokinetic purposes on selected groups. These measurements are affected by substrate transit, which in turn depends on diet, position of the subject, disease, and drug effects. To evaluate these influences, GI emptying and intestinal transit can be evaluated in parallel, using orally administered sulphur colloid or diethylenetriamine-pentaacetic acid. These probes are labelled with technetium-99 m (Sn-catalysed) or indium-111. Gallium-67 citrate is also useful (American College of Radiology, 2015). These non-absorbed, pharmacologically inert materials are combined in the test diet as markers for transit and emptying. Although these studies are completed using scintigraphy, a real time tomographic study (SPECT or PET) can be conducted using a similar protocol, and the bio-distribution and

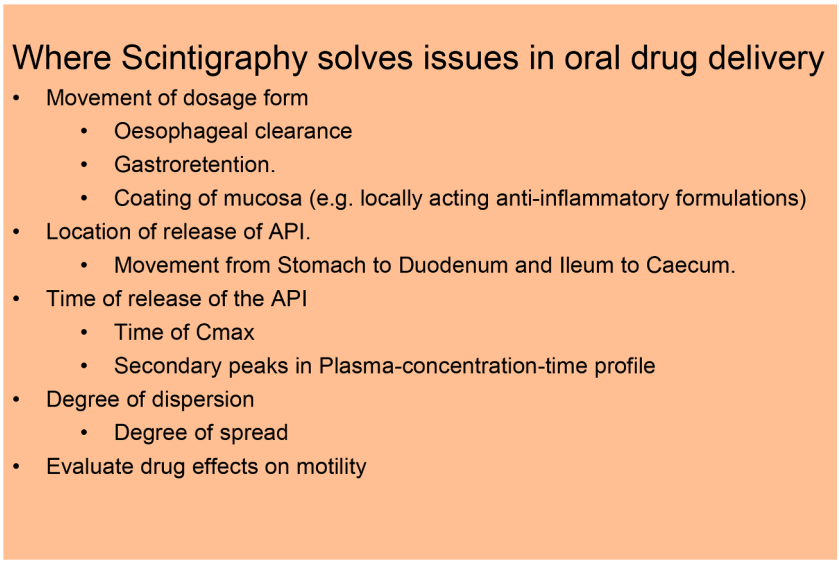

Fig. 8.5. The employment of gamma scintigraphy in oral drug delivery research.

disposition of the drug or drug formulation could be assessed.

8.2.7. Drug delivery

From the first formulations in the 17–18th century, a substance with known effects was desired to be shelf stable, to be safe and to be effective. Today, these remain important goals. Dosage forms have become sophisticated especially with the employment of nanotechnology to engineer more precisely targeted drug delivery systems. The objective is now to design delivery systems that are spatially and temporally efficient: maximising delivery with decreased off-target binding, decreasing drug degradation, and attaining of precise drug levels at the target over the appropriate period.

The attraction of nuclear medicine is that all these aspects can be evaluated. Imaging can follow the core of the delivery system, the drug, or any compartment of the delivery system, if they can be stably labelled with a radioisotope. Man and colleagues have considered the stability of radiolabelled liposomal formulations for nuclear medicine imaging. They observe that radionuclides used for labelling may have the same deposition pattern as the liposome, confounding interpretation (Man et al., 2019). They review the qualitative distribution of many of the commonly used radionuclides and show that small differences in the radiolabelling methods lead to redirection of the preparation. SPECT has the advantage of follow more than one isotope, if they have non-overlapping spectral features then this may help to overcome some of these limitations. (Fig. 8.4).

Using these methods, the stability of the delivery system, and the fate of the contents in real time, enables the investigator to measure the effectiveness of the delivery system. These very sophisticated approaches are often employed in oncology or to access inoperable spaces whereas oral delivery utilises less sophisticated approaches. Nevertheless, there are many components, in drug delivery, that cannot be easily inferred by studying drug levels in biological fluids, especially with regard to transit, distribution and regional absorption.

8.2.8. Nuclear medicine and oral drug products

The oral route supports independent therapy management provided that the dosage form is easy to swallow. Drug substances can be irritant to the oesophagus and injury to the unprotected squamous epithelium is exacerbated by acid reflux. We accept that drug design and formulation require good *in vitro* and *in silico* prediction methods during development (see for example Section 1-4, and 7–8 of these series), but also there is sometimes a need to evaluate key attributes of the formulation including mucoadhesive properties and GI transit profile. This may be required to avoid unwanted mucoadhesion or an inappropriate transit profile for a given formulation (Drumond and Stegemann, 2018). Gamma scintigraphy has been used successfully to determine oesophageal transit times of solid oral dosage forms (SODF) since the early '90′s (Wilson, 1994), allowing transit profiling of formulations along the proximal, distal, and lower regions of oesophagus (Wilson et al., 1988) and has been identified as the 'gold standard' method to successfully detect differences amongst distinct SODFs in this respect (Perkins et al., 1999). Because food and drug formulation can be labelled separately, the impact of fed or fasting conditions can readily be monitored using technetium-99 m to label the food matrix and indium-111 to label the formulation (Davis et al., 1984). As previously mentioned, transit, distribution and regional absorption of drug formulations are often critical to performance. Fig. 8.5 summarises some of the key advantages of scintigraphy in this respect.

Drugs that have a short absorption 'window' in the GI tract will have poor absorption, including anti-viral agents, bisphosphonates, vitamins, and many BCS Class II & IV compounds. For these drugs, gastroretentive drug delivery systems offer the advantages in prolonging the gastric emptying time ((Agyilirah et al., 1991) and nuclear imaging has been employed in investigating their properties. The formulations are designed to prolong the time of release immediately above the most absorptive segment of the gut, the duodenum, for example a floating dosage technology (Fell and Digenis, 1984). Note that many large dosage forms are retained in the stomach for a long period and thus in vitro demonstrations of floating *per se* may not translate to the ascribed mechanism (Burke and Wilson, 2006). Nevertheless, it has been widely employed; for example, the study of Ma and colleagues, where alginate-based floating and non-floating microspheres were labelled with ^{99m}Tc and fed to volunteers (Ma et al., 2008). Chitosan was added to the gelation medium increasing diltiazem incorporation in the optimised formulation. The scintigraphy confirmed that the optimised floating sustained-release microspheres significantly prolonged gastric retention time, compared with non-floating microspheres. Similarly, Pund and colleagues prepared a tableted rifampicin formulation from carbopol and HPMC formulation which showed gastric retention beyond 6 h in fasted subjects (Pund et al., 2011). Overall, this approach is heavily influenced by the physiological differences in the fed and fasted state, which are so difficult to overcome and the employment of more than one mechanism simultaneously to achieve gastroretention is suggested (Tripathi et al., 2019). As can be noted, 'recent' developments in the field have a long lineage back to previous studies and awareness of the path of development is important as new data rarely supplant old

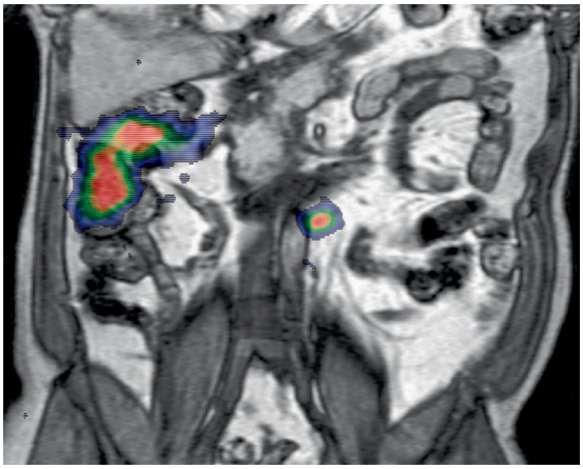

Fig. 8.6. Combined magnetic resonance (MRI) (gray shades) and gamma camera image (colour) of a delayed release device with released contents at the top of the ascending colon in a volunteer (Wilson 2012). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observations in this branch of biopharmaceutics.

The distal gut is difficult to explore and makes systems of targeted delivery based on microbial fermentation of a matrix or coating difficult to detect. Sharma and colleagues showed dispersion of a technetium-labelled tinidazole in a guar gum-based formulation a in the transverse and descending colon (Sharma et al., 2016). From the image provided in the article, this was probably too late to achieve effective colon targeting. A later study using a fenugreek-based polysaccharide to deliver metronidazole seems to have been more successful although there was some indication of early release. This is probably a more realistic goal - allowing some loss prior to traversing the ileocaecal valve (Sharma et al., 2018).

8.2.9. Future perspectives: smaller detectors with more utility

Combination of imaging modalities, particularly those with a smart phone interface, become more popular in diagnostic applications involving sonography and to new extent, nuclear medicine. An Italian group described ideas for combining ultrasound and a gamma camera more than ten years ago (Meo et al., 2009). The concept was to get morphological and functional information simultaneously, although field of view was limited and the device went through cycles of improvement (Pani et al., 2011). The idea was followed as a basis for a breast-screening device (O'Connor, 2014). Simple multimodality by rushing a subject from the MRI suite to the gamma camera has been attempted by our group as shown in Fig. 8.6 but it is hardly practical to tie up two expensive clinical instruments in routine formulation research.

The refinement of detectors based on cadmium telluride semiconductors encouraged the development of restricted matrix, handheld detectors, which bridge the gap between clinical and small animal imaging (Knoll et al., 2014). The relative advantages of scintillation probes with a smaller field of view based on a crystalline detector and semiconductor devices have been explored in radioisotope guided surgery (Povoski et al., 2009). The scintillation probes tend to have bulkier handsets, and are more sensitive to medium and high-energy radionuclides, but have poor scatter rejection.

A hybrid optical and gamma camera system has been clinically evaluated (Alqahtani et al., 2018). The handheld camera uses a thallium-doped caesium iodide CsI(TI) columnar scintillator fitted with a pinhole collimator aligned with an optical camera. Images are fused to combine external anatomical features with the scintiscan. The data was recently demonstrated at an UNGAP workshop in Greifswald and images of the thyroid of a patient dosed with iodine-123 compared in conventional and novel gamma cameras.

MRI sequential or simultaneous registration has been recently introduced to improve interpretation of the images and enables the recording of additional functional and metabolic features. Applications of multimodality have developed beyond encompassing micro-CT to add PET and SPECT signals, along with bioluminescent and/or fluorescent label detection in the same experiment. This scenario increases exponentially the research output, decreasing the number of experimental manipulations and the number of animals used whilst increasing the longitudinal power of each experiment.

8.2.10. New imaging agents and techniques

Therapeutic agents may display poor permeability characteristics but have exquisitely targeted sensitivity. In this situation, utilising imaging radionuclides and following the biodistribution by SPECT and PET can be used, especially with the short-lived tracers ^{18}F and ^{64}Cu. These tracers have half-lives of 1.83 and 12.7 h, respectively. They have been employed to follow small interfering RNA constructs (Wang et al., 2016). Kim and colleagues combined confocal imaging, tissue sampling and PET to follow the accumulation of (^{64}Cu)-labelled poly-glucose nanoparticle into tumour associated macrophages (Kim et al., 2018). The advantage of radionuclide tracers compared to quantum dots is the ability to show deep tissue permeation (Franc et al., 2020).

A Cu-Fe-Se nanosheet drug carrier system has been described that exploits the ability to label the doxyrubicin-loaded nanosheets with technetium-99 m and follow the distribution by SPECT in a tumour bearing mouse (Jiang et al., 2018).

Radioisotopes do offer the opportunity to share the same chemistry in manufacture of radiopharmaceuticals taking advantage of the different half-lives according to need. An experimental approach based on this concept would be the combination of ^{64}Cu, a positron emitter or the beta emitter ^{67}Cu. The longer half-life (2.58 days) and high energy (β-max,562 keV) of ^{67}Cu makes it suitable for a therapy and SPECT use (Hao et al., 2017). In this article, pertuzumab, a HER2 specific antibody, was conjugated with the bifunctional chelator 2-S-(4-isothiocyanatobenzyl)−1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA-NCS), and radiolabelled with ^{67}Cu.

Tran and colleagues synthesised (^{123}I)- fenofibrate and used SPECT/CT to follow the distribution in rats when delivered in (^{125}I)- triolein. This approach enabled elegant proof of co-localisation and exploration of different profiles of the drug when administered in suspension and in a lipid-based formulation (Tran et al., 2020).

8.3. Conclusions

Manufacturers have reported developments in gamma detection devices, with a wide range of energy detection from 50 up to 1200 keV, expanding the possibility of monitoring not only SPECT tracers but also simultaneously different PET markers (Beekman et al., 2021). In addition, the use of radiopharmaceuticals in minute amounts ensures no pharmacological effects attributable to the marker, making this technology superior. The availability of detection systems able to use more the one detection technology has revolutionised drug research at clinical and more especially at preclinical stages (Wu and Shu, 2018). More importantly, advances from drug research are combined with advances in nuclear medical imaging in better-targeted surgery (Van Oosterom et al., 2019). Ge and co-workers have reviewed current approaches for radiolabelling nanomaterials in multimodality with emphasis on cancer therapy. They highlight four key factors that should be kept in mind when designing new probes. These are stability of the radiolabel and avoiding radionuclide shedding from the radiopharmaceutical, safety, the need for simple procedures in final nanomaterial assembly and finally a good appraisal of multimodality imaging by balancing the sensitivities of the various methods (Ge et al., 2020).

Nuclear medical applications for oral drug delivery research are well

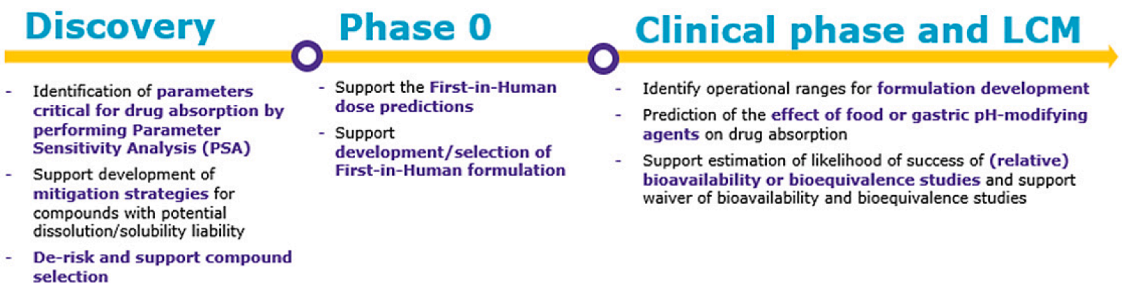

Fig. 9.1. Phase-dependant questions relevant in the formulation development process which can be addressed by an in silico absorption model.

established; however, the instrumentation is expensive, and, in most situations, availability restricted. Innovations based on small portable detectors are appearing on the horizon and could provide an extension for drug delivery scientists for informative, image-guided research protocols. These inputs provide a more integrated information base which supports physiologically biopharmaceutics based modelling and can be applied to complex molecules. Evaluation of key patient groups is essential in that section of the population where development of new medicines is being pursued. This especially applies to the paediatric therapy where the microdosing and imaging data obtained opportunistically, charting development of the oral absorption process, is especially useful in POP -PK and PBPK modelling. This has been a key activity in UNGAP and in the preceding OrBiTo European initiatives but naturally comes towards the ends of the activities when data has been collected and considered. Because this is still an ongoing activity, the next two segments provide a review of these approaches, contributed by our industrial and our academic colleagues.

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9. *In silico* drug absorption modelling to support formulation development

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- Physiologically-based biopharmaceutics modelling (PBBM) approach is being currently used by the pharmaceutical industry and employs compound intrinsic properties, as compound's solubility and possible precipitation behaviour, to predict its pharmacokinetic behaviour. This information aids during decisions on compound form, or formulation, and also helps to manage drug risk assessment by providing information on drug-drug interactions, amongst other parameters.
- Despite PBBM is still in its infancy, research in this area has been increased recently, and new procedures as FirstinHuman prediction and iterations of the "building, verification, and approval cycle" model have been proposed, as well as the use of data obtained from biorelevant conditions of solubility, and pH, amongst others.
- Opportunities for improvement of predicting oral absorption were already identified.

Summary

A holistic approach to investigate the possible in vivo behaviour of chemical entities is to apply Physiologically-Based Biopharmaceutics Modelling (PBBM). By combing compounds parameters, formulation parameters and physiological parameters the predictions can be used to establish a first understanding of new compounds within the pharmaceutical development process or to deepen the understanding of already better characterised active pharmaceutical ingredients (APIs) in context of oral absorption. Parameter Sensitivity Analysis (PSA) is within the PBBM approach a useful tool to investigate sensitivity of absorption predictions towards changes of defined simulation input parameters. PBBM is addressing the need to increase the number of simulation approaches within the pharmaceutical development process and is increasingly used by pharmaceutical companies.

9.1. *Introduction*

The PBBM approach is strongly supported within the pharmaceutical industry (Heimbach et al., 2019). The concept is linking a compound's physico-chemical properties, formulation characteristics, and physiological conditions to its pharmacokinetic behaviour (Bermejo et al., 2020; Mitra et al., 2020). An essential part of the PBBM approach is to address questions and hypotheses raised in context of the compound's absorption after oral administration of a drug product (DP). By assuming a direct connection of the compound's *in vivo* dissolution and absorption, the questions addressed by PBBM are therefore mainly related to the *in vitro* dissolution of the compound which is, in the optimal case, bio-predictive. As dissolution is closely related to the compound's solubility and possible precipitation behaviour, these parameters are also important in the PBBM concept. Questions addressed by absorption modelling are dependant on the phase of development and increase in complexity at later development stages due to increasing number of covariates e.g. administration of DP under fed conditions.

9.2. *In silico drug absorption modelling – opportunities in drug development*

Especially at early development stages, absorption modelling is applied to assess the risk associated with variations in the compound form (e.g., parent form vs. salt or co-crystal) or formulation (e.g., powder in capsule formulation vs. immediate release tablet formulation vs. modified release tablet formulation). Predictive *in silico* tools are in this context used to facilitate data driven decision-making when different formulation options are available by providing first insights in the potential DP's *in vivo* behaviour. As the input parameter for these early and simple exploratory predictions vary within the pharmaceutical industry and are also compound specific, there is no clear guidance or a "best practice" document available describing the early risk assessment procedure in detail. In an industry setting as described by Kesisoglou, a structured early risk-assessment is performed by conducting a Parameter Sensitivity Analysis (PSA) on parameters possibly influencing the absorption. By varying the input for the parameter of interest in a relevant range, possible factors limiting oral absorption can be identified and mitigation strategies for formulation development can be developed (e.g., by developing a drug delivery system). Parameters which are typically investigated at early stages in a PSA are e.g., solubility, permeability, precipitation behaviour, particle size, and dose.(Kesisoglou, 2014)

Besides the application of *in silico* absorption modelling in early "risk assessments", the concept is meanwhile also applied in the later stages of formulation development on a regular basis. While *in silico* tools are already widely accepted in context of predictions of drug-drug interactions by regulatory agencies, the confidence in prospective absorption predictions is still rather low (Darwich et al., 2017a;

Margolskee et al., 2017; Zhao et al., 2012). To increase confidence in prospective absorption predictions, a lot of academic and industry research is dealing with this topic and the number of scientific publications addressing prospective absorption predictions is consistently increasing (Hens et al., 2018; Kambayashi et al., 2019; Tistaert et al., 2019; Sjögren et al., 2016).

In context of connecting early absorption predictions of the preclinical development phase with questions arising in the clinical formulation development setting, the prediction of the First-in-Human (FiH) dose is of special importance. Miller et al. recently suggested a cross-industry strategy for conducting FiH predictions and presented a proposal for the switch of absorption predictions from preclinical species towards human predictions (Miller et al.,2019).

In general, for model informed formulation development, it is necessary to apply the model "building, verification, and application cycle" in an iterative procedure to address more complex questions compared to the PSA in the preclinical development phase.

9.3. Opportunities for improvement identified

Special emphasize is currently given to the predictive performance of *in silico* tools in prospectively predicting formulation changes for e.g., support of waiving relative bioavailability trials or bioequivalence studies, predicting food effects and the effects of increased gastric pH (or both in combination) on the *in vivo* absorption (Aburub et al., 2019; Mitra et al., 2020; Parrott et al., 2020; Gesenberg et al., 2019; de Waal et al., 2020; Riedmaier et al., 2020).

Actual publications are frequently identifying areas of further improvement in the software packages. For increasing confidence in prospective predictions, the OrBiTo consortium suggests using e.g., biorelevant solubility data instead of solubility determined in simple buffer solutions to increase model predictivity (Darwich et al., 2017). A decision tree for supporting the selection of the dissolution model being most bio-predictive based on physico chemical compound properties and formulation characteristics is also published by the consortium and can be used to inform the absorption model with most bio-predictive *in vitro* release data (Andreas et al., 2018). A recently published position paper from the OrBiTo consortium also presents case examples on the integration of *in vitro* dissolution data into PBBM tools to predict in vivo DP behaviour of various dosage forms (Jamei et al., 2020).

Special emphasis is also given on the representation of the human physiological conditions when the absorption model is used for predicting the effect of food or the influence of increased gastric pH. Li et al. identified the dynamic decrease of pH over time in the fed stomach as a possibility for improvement of prospective food effect absorption predictions (Li et al., 2018). Parrott and colleagues used the concept of PBBM to identify the role of self-buffering and acidulant effects of a tyrosine kinase inhibitor after oral administration. The authors emphasized that current commercially available absorption models do not include separate description of surface and bulk pH which can be of relevance by simulating the *in vivo* absorption of weakly basic drugs (Parrott et al., 2020).

9.4. Conclusion

The concept of PBBM and applying prospective absorption predictions is of increasing importance in the process of decision making in context of the formulation development. Risk assessments and hypothesis testing can be supported without pre-clinical and clinical studies being necessary which is in line with the 3R principles: Replace, reduce, and refine (Russell and Burch, 1959). The application of the PBBM concept is also streamlining the drug development. Especially when accelerated timelines are deserved due to e.g., designation of an API as breakthrough therapy by the U.S. Food and Drug Administration (FDA), the PBBM concept is supporting the development process.

The interest in prospective absorption predictions is also a topic of increasing relevance for regulatory agencies. This can be perceived by the increasing number of workshops addressing the topic of absorption modelling (Pepin et al., 2021; Wagner et al., 2015; Zhang et al., 2017). Nevertheless, *in silico* models used for prospectively predicting *in vivo* absorption of new active pharmaceutical ingredients are strongly dependant on the input parameters characterizing the drug substance. Therefore, *in silico* absorption models should always be used in context of the holistic PBBM approach integrating data from most bio-predictive *in vitro* assays. By combining PBBM with the population pharmacokinetic/pharmacodynamic (pop-PK/PD) approach will further streamline the drug development process by making use of data driven decision making processes.

10. Pharmacometric modelling of oral drug absorption

By Adam S. Darwich, KTH Royal Institute of Technology, Stockholm, Sweden, and Leon Aarons, The University of Manchester, United Kingdom

- Extreme treatment outcomes, which are far from the mean drug behaviour, are determinants of drug safety, and are difficult to predict. The population pharmacokinetic/pharmacodynamic (popPK/PD) approach is applied to study drug response and its variability in a population sample.
- The popPK/PD approach depends on experimental and clinical data and relies on drug kinetic models and statistical models, from where fixed and random effects are estimated. In this process pharmacokinetic parameters, demographics, biomarkers and other factors are taken into account. The approach is used to characterise therapies, dosage regimens, and stratification to relevant patient populations and scenarios.
- PopPK/PD methods complement PBPK modelling by allowing estimation of variability and covariate effects.
- Several case studies where popPK/PD methods have been used are described in this chapter.

Summary

It is extreme events that are the most perilous; the tropical storms in the Caribbean and bushfires in Australia become landmarks of climate change. In clinical pharmacology, it is the patients that experience the extreme treatment outcomes that are at the greatest risk of severe adverse drug reactions, or failure of treatment due to inadequate response (Katz and Brown, 1992; Darwich et al., 2017b; Holford and Buclin, 2012). Many of the processes involved in oral drug absorption are variable, even stochastic, and cannot be anticipated by mean projections alone (Welling and Tse, 1984). Therefore, the ability to describe and explain variability is at least as important as understanding the mean behaviour of orally administered drugs.

10.1. Introduction

The population pharmacokinetic/pharmacodynamic (pop-PK/PD) approach provides a methodology for studying drug response and its variability in a population sample. This is typically accomplished using models of drug kinetics combined with statistical mixed-effects modelling, where fixed and random effects are estimated for the model input parameters along with residual variability. Where possible, explanations of the random effects are sought through studying the association between pharmacokinetic parameters (such as clearance, absorption and volume of distribution) and other factors, such as patient demographics, biomarkers and more (Aarons, 1991). This approach is dependant on observations and experimentation, obtained through controlled clinical trials. However, the discipline rests heavily on the principles and mechanisms defined by pharmacokinetics and pharmacodynamics. More in-depth technical reviews and tutorials can be found in for example (Bonate, 2005; Mould and Upton, 2012; Ette and Williams,

2004).

10.2. Pharmacometric modelling

The pop-PK/PD approach aims to characterise therapies, at relevant doses, in relevant patient populations. By describing and understanding variability in and between populations and other key variables, we can minimise the invasiveness of clinical studies in patient populations and define more optimal dosage regimens (Aarons, 1991). Over the past five decades, these methods have gained wide use in drug development to inform clinical trial design, dose selection through the study of efficacy and toxicity, formulation and food effects, drug interactions, disease effects, and much more (Williams and Ette, 2000).

The population approach provides a useful tool for studying variability in drug absorption, investigating explanatory variables and/or mechanisms. Here follows a discussion on the relevance of pop-PK modelling for current and future research in biopharmaceutics, advantages and limitations of the method and how it informs other approaches.

10.2.1. Accounting for variability in drug absorption

Ideally, the effect of a drug could be measured entirely using clinical endpoints and validated PD biomarkers. It is however well recognised that PK is an important source of variability in drug response, and considering our current limited understanding of variability in PD, PK is perhaps the most important determinant of *explainable* variability (Lin, 2007). Therefore, plasma/whole blood drug concentration-time profiles are often the *de facto* surrogate of drug response.

In PK, for orally administered drugs, bioavailability and absorption are important factors in determining interindividual and interoccasion variability. These are indeed the first of several steps determining drug exposure over time in the ADME process: absorption, distribution, metabolism and elimination (Welling and Tse, 1984; Dressman et al., 2008). However, relatively few measurements are taken at early times after drug administration. Herein lies the challenge with describing oral drug absorption, be it using 'top-down' pop-PK, informed mainly by clinical data, or fully mechanistic methods, such as 'bottom-up' physiologically based pharmacokinetic (PBPK) modelling, that rely heavily on physiological data and *in vitro* characterisation of compound properties.

In the absence of clinical data, PBPK modelling offers a compelling method for describing these processes, using mechanistic equations and inputs translated from *in vitro* experiments and pre-clinical species (Jones et al., 2015). However, with model complexity comes uncertainty and bias that is difficult to overcome through estimation methods using conventional venous drug concentration-time data (Nestorov, 2003). It is also challenging to accurately anticipate and describe observed *in vivo* variability in concentration-time profiles using *a priori* fixed probability distributions in input parameters. Therefore, we often observe that PBPK models struggle with describing *in vivo* variability in PK, between individuals and occasions.

Classical pop-PK takes an empirical approach to modelling, letting the clinical data guide the structure of the model and estimation process. Even within this realm of data-driven approaches however it is possible to define "absorption agnostic" approaches which use purely empirical functions or parameterisations of absorption processes, and "biopharmaceutically focused" approaches where more use is made of all available data (*i.e.,* going beyond the dataset being examined) to describe and understand absorption more mechanistically. In both cases the aim is to describe absorption and its variability accurately and explore associated factors.

Combining mechanistic models, such as PBPK, with mixed-effects modelling, has increased in use over the last decade (Tsamandouras et al., 2015a). This means that we can combine physiological and drug/formulation-specific data as priors with mixed-effects parameter estimation in a 'middle-out' approach. The old argument that pop-PK is used for interpolation and PBPK for extrapolation, therefore no longer holds true.

10.2.2. Modelling complex oral absorption in a population pharmacokinetics paradigm

A wide variety of models have been applied in pop-PK to describe absorption, varying in degree of complexity and mechanism. Assorted examples are given in Table 10.1: from classical first and zero-order rate of absorption, with or without lag time, to mixed absorption models, transit models and more (Holford et al., 1992; Savic et al., 2007). The choice of model complexity is driven by the data, model performance and the purpose of the analysis. The availability of data, sampling points in the absorption phase and number of study participants will also often determine what models are feasible to implement, while the specific choice of mechanism is often driven by the research question, hypothesis or purpose of the project (Mould and Upton, 2013).

Modelling of oral bioavailability can be done using several different approaches, and at different levels, depending on the available data and information about the drug. By combining oral and intravenous drug concentration-time data the absolute oral bioavailability can be determined, along with its variability (Variol et al., 2002). Bioequivalence data of oral formulations can be used to estimate the relative bioavailability. In the absence of any comparators, the variability in oral bioavailability can be estimated alone Bjugard Nyberg et al., 2020) which can be a useful potential explanation of covariance in apparent oral clearance and volume of distribution. Further, mechanistic features, such as the well-stirred liver model, can be incorporated to describe first-pass extraction in the liver (Dooley et al., 2015).

10.2.3. Case studies

Here follows a description of a few illustrative case examples to highlight the practical use of pop-PK for absorption modelling. Of note is that absorption-related effects are rarely studied in isolation, as the model-based approach allows us to investigate multiple factors.

10.2.4. The effect of formulation, metabolic genotype and metabolic drug-drug interactions on oral absorption and bioavailability

Darifenacin is an antimuscarinic agent for the treatment of overactive bladder disease. The drug is mainly metabolised through hydroxylation via cytochrome P450 (CYP) 3A4 and CYP2D6 (Skerjanec, 2006).

Kerbusch, and co-workers, analysed clinical data from 18 studies, including intravenous, oral solution, immediate-release, and several controlled release formulations. The analysis aimed to examine variables relevant for explaining variability in darifenacin PK. A darifenacin parent-hydroxy metabolite model was developed, describing metabolic first-pass formation through splitting the oral darifenacin dose into absorbed unchanged parent and metabolite formed on the first-pass. Both parent and metabolite disposition was described using a two-compartment model with first-order absorption and linear clearance (Kerbusch et al., 2003).

Both oral bioavailability and the absorption rate constant were found to depend on the type of oral formulation. Further, oral bioavailability of the parent drug was dependant on formulation, dose, CYP2D6 genotype and interactions with CYP inhibitors ketoconazole and erythromycin (Kerbusch et al., 2003).

10.2.5. Interaction between food and formulation effects on oral absorption

DRL-17,822 is a cholesteryl ester transferase protein inhibitor currently in clinical development. The candidate drug exhibits a high lipophilicity with a logP of 8.86, suggesting low solubility, high bile-mediated solubilisation and impact of food. A nanocrystal formulation showed large effects of concomitant food intake. Hence an amorphous solid dispersion formulation was developed (Kruithof et al., 2019).

Goulooze, and co-workers, developed a two-compartment pop-PK model with linear elimination, a six-transit compartment model and

Table 10.1
Examples of absorption models implemented in population analysis of clinical data.

Level of mechanism and complexity	Model	Description and use cases
Mechanism: low, complexity: low	k_a[1]	• First-order absorption rate. • Often relevant in most cases of readily absorbed drugs formulated as oral solutions and immediate-release. • Simplicity allows modelling of sparse datasets.
Mechanism: low, complexity: low	k_0[1]	• Zero-order absorption rate. • May be relevant to describe saturable absorption processes or controlled-release formulations.
Mechanism: low, complexity: low	t_{lag}[2]	• Absorption process with lag-time. • Describes delay in initiation of absorption due to various processes, such as gastric emptying, release from formulation, delay in reaching observed compartment etc.
Mechanism: low, complexity: medium	Mixed 0–1st order absorption[1]	Sequential zero and first-order absorption. Produces delayed absorption with a mixed behaviour.
Mechanism: low, complexity: medium	Transit models[3]	• Using Erlang model with fixed number of transit compartments or estimate number of transit compartments. • Produces delayed absorption input.
Mechanism: low, complexity: medium	Split dose models[4]	• The dose is split into two or more depots, can combine zero/first-order absorption rate with lag-time. • Multiple absorption windows, multiple peak data.
Mechanism: low, complexity: medium	Flexible input functions[5,6]	• For example, using inverse Gaussian functions, splines, polynomials and more. • Describe complex absorption profiles, for example with multiple peaks.
Mechanism: low, complexity: medium	Michaelis-Menten (M-M) or E_{max} absorption[1,7]	• Saturable absorption or efflux, due to for example transporter effects, saturable first-pass metabolism or other processes.
Mechanism: medium, complexity: medium	Gastric emptying[8]	• Mechanistic description of gastric emptying process, where stomach acts as depot for drug absorption. • Observed lag time or multiple absorption phases. Allows mechanistic inferences about gastric emptying.
Mechanism: medium, complexity: medium	Enterohepatic circulation[9]	• Enterohepatic circulation with intermediate depot. Can be implemented at different levels of mechanism. • Describe late peaks in concentration-time profiles or plateaus in elimination phase.
Mechanism: medium, complexity: medium	Mechanistic dissolution[10]	• Describes dissolution limited absorption using mechanistic models such as Noyes-Whitney, Wang-Flanagan and more. • Use of prior dissolution data to inform model development.
Mechanism: high, complexity: high	Fully mechanistic absorption model[11]	• Physiologically-based model, typically based on the compartmental absorption and transit model. • Study complex formulation or gut effects to make inferences about mechanisms.

[1] Holford et al., 1992.
[2] Nerella et al., 1993.
[3] Savic et al., 2007.
[4] Jin et al., 2014.
[5] Brvar et al., 2014.
[6] Margolskee et al., 2016.
[7] Svenson et al., 2018.
[8] Ogungbenro et al., 2011,
[9] Sherwin et al., 2012.
[10] Tsamandouras et al., 2015b.
[11] Olivares-Morales et al., 2016.

relative bioavailability to describe the absorption of DRL-17,822 and to study the complex interaction between food and formulation effects. The authors were able to quantify the impact of food and formulation on DRL-17,822 absorption to potentially inform further clinical study design and future dosing guidance (Goulooze et al., 2020a).

10.2.6. The effect of disease and prokinetics on gastric emptying using paracetamol as a probe

Gastric emptying is important as a rate-limiting step for the absorption of nutrients and drugs. Enteral feeding is important for the maintenance of critically ill patients. However, a proportion of these patients develop intolerance, resulting in delayed gastric emptying and poorer outcomes, and therefore prokinetic therapies, such as erythromycin and metoclopramide are used. Paracetamol is a rapidly absorbed drug, where the rate of absorption is limited by gastric emptying. The drug is therefore used as a probe of gastric emptying function (Willems et al., 2001; Ogungbenro et al., 2011).

Ogungbenro, and co-workers, developed a semi-mechanistic pop-PK model of paracetamol, describing its gastric emptying into the small intestine and systemic absorption in order to study the effects of prokinetics, metoclopramide and erythromycin, on gastric motility. The modelling approach was able to determine the magnitude of effect of prokinetics on gastric emptying half-life in critically ill patients intolerant to enteral feeding. Further, the study provides an approach for the development of newer agents and the optimization of study design [Ogungbenro et al., 2011].

10.2.7. Transporter drug-drug interactions and their effect on oral bioavailability

Talazoparib is an orally bioavailable small molecule used in the treatment of BRCA-mutated human epidermal growth factor receptor 2-negative locally advanced or metastatic breast cancer. The drug is mainly eliminated through renal excretion and is a substrate of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). Data from four clinical studies were used to develop a population pharmacokinetic model of talazoparib to investigate any potential covariates that may inform dose recommendations of the treatment. The pop-PK analysis found that concomitant use of potent P-gp inhibitors, such as itraconazole, resulted in an approximately 50% increase in relative oral bioavailability and exposure, which was clinically relevant and therefore recommended a lower dose in these cases (Yu et al., 2020).

10.3. Conclusions. current opportunities and future direction

Oral drug absorption and biopharmaceutics effects have been extensively modelled using population approaches with a range of models with varying degrees of mechanistic specificity. The differences between traditional pop-PK with empirical or compartmental models and PBPK modelling have diminished as we see a wide variety of applications where increasingly mechanistic modelling is combined with population approach mixed-effects modelling. The selection of an overall approach is therefore much more multi-faceted and dependant on questions, such as availability of data and drug information, purpose of the modelling analysis and more.

Pop-PK analysis allows a model-based approach to investigate

Table 10.2
Current and emerging trends in pop-PK modelling for drug absorption.[1].

Well-established areas of use	Emerging uses
• Inter-individual variability • Formulation effects • Food effects • Gastric emptying • Drug-drug interactions • Transporter effects • Genetic polymorphism • Inter-occasion variability • Optimal design	• Combined 'middle-out' approaches: including transfer of mixed-effects methods into PBPK domain, and vice versa. • Deconvolution and IVIVC • Combining pop-PK with big data and machine learning, • 'Virtual bioequivalence'

[1]references cited in the main body text.

clinical outcomes and findings. This allows multiple variables to be explored and readily links to pharmacodynamic measurements. Over the past decade, the natural sciences have seen the emergence of large datasets including 'omics data, such as genomics and proteomics (Couto et al., 2020; Singh, 2019). We are also seeing the emergence of real-world data, large-scale heterogenous datasets that allow us to study drug effects closer to clinical practice (Goulooze et al., 2020b; Wang et al., 2020). As has been seen in the literature, pop-PK can be used together with machine learning to leverage big data (Ribbing et al., 2007). Models of machine learning may also be used together with more traditional pop-PK analyses to build ensemble models with increased of predictive performance (Poynton et al., 2009).

Apart from novel 'omics and big data, recent initiatives, such as the IMI (Innovative Medicines Initiative) OrBiTo (Oral Biopharmaceutics Tools) project, have led to the generation of novel measurements in humans, such as *in vivo* dissolution profiles, and more complex *in vitro* experiments to measure dissolution processes and absorption (Butler et al., 2019). Due to the natural variability in these types of datasets, mixed-effects modelling lends itself well the analysing, accounting for variability and random error and thereby reducing biases in estimations of parameters and effects (Hing et al., 2001).

Further, there has been a lot of activity in the modelling sciences applied in the areas of bioequivalence and *in vitro-in vivo* correlation (IVIVC) of formulated dissolution profiles (Table 10.2). Here the population approach allows analysis of interindividual and inter-occasion variability in datasets to maximise learning and improve study design (Shin et al., 2020).

Population pharmacokinetics can be considered a mature discipline by now, with certainly the fundamental principles having remained largely established and consistent for decades. However, novel methods for data gathering, the emergence of 'omics and big data, together with mixed modelling approaches incorporating increased mechanistic detail into analysis and interpretation, has led to new areas of application in the field of biopharmaceutics. For the purpose of describing and explaining sources of variability, mixed-effects modelling and the population approach remain important methodologies in absorption modelling.

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