Strathclyde Institute of Pharmacy and Biomedical Sciences

IN VITRO AND *IN SILICO* PROFILING TO ASSIST PHARMACEUTICAL DEVELOPMENT

By

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List of abbreviations

5-FU, 5 Fluorouracil

ACAT, Absorption Compartmental And Transit

ACD, Advanced Chemistry Development

ADAM, Advance Dissolution Absorption Metabolism

ADME, Absorption Distribution Metabolism Elimination

ADMET, Absorption Distribution Metabolism Excretion and Toxicity

ANOVA, Analysis Of Variance

APIs, Active Pharmaceutical Ingredients

ASD, Amorphous Solid Dispersion

AUC, Area Under the Curve

BA, Bioavailability

BCRP, Breast Cancer Resistant Protein

BCS, Biopharmaceutics Classification System

BE, Bioequivalence

BMI, Body Mass Index

BP, British Pharmacopoeia

BS, Bile Salt

CAT, Compartmental And Transit

CES, CarboxylEsterase

C_{max}, Maximum Concentration

CMC, Critical Micelle Concentration

CRUK, Cancer Research UK

CYP, Cytochrome P450

DCS, Developability Classification System

DDI, Drug Drug Interaction

DLT, Dose Limited Toxicity

DoE, Design Of Experiment

DR, Dissolution Rate

EFPIA, European Federation of Pharmaceutical Industries and Association

EMA, European Medicine Agency

F, Fraction Absorbed

FaHIF, Fasted Human Intestinal Fluid

FaSSGF, Fasted State Simulated Gastric Fluid

FaSSIF, Fasted State Simulated Intestinal Fluid

FDA, Food and Drug Administration

FeHIF, Fed Human Intestinal Fluid

FeSSGF, Fed State Simulated Gastric Fluid

FeSSIF, Fed State Simulated Intestinal Fluid

F_{up}, Fraction Unbound in Plasma

GC, GlycoCholate

GI, Gastro-Intestinal

GIT, GastroIntestinal Tract

GSE, General Solubility Equation

HCL, Hydrochloric Acid

HIF, Human Intestinal Fluid

HPLC-UV, High Performance Liquid Chromatography - Ultra-Violet

IMI, Innovative Medicine Initiatives

IR, Immediate Release

IVISIVC, In vitro In silico In vivo Correlation

IVIVC, In vitro In vivo Correlation

IVIVE, In vitro In vivo Extrapolation

K_a, Absorption rate constant

K_e, Elimination rate constant

K_m, Michaelis constant

MAD, Maximum Absorbable Dose

MDCK, Madin-Darby Canine Kidney

MHHE, Modified Henderson Hasselbalch

mM, millimolar

MO, Mono-Olein

mpe, mean percentage error

MRPs, Multiresistant Proteins

MTD, Maximum Tolerated Dose

MW, Molecular Weight

NaTC, Sodium Taurocholate

NCEs, New Chemical Entities

OA, Oleic Acid

Orbito, Oral Biopharmaceutics Tools

PAMPA, Parallel Artificial Membrane Permeability Assay

P_{app}, Apparent Permeability

PBPK, Physiologically Based PharmacoKinetics

PCA, Principal Component Analysis

pe, percentage error

P_{eff}, Effective Permeability

PEG, PolyEthylene Glycol

P-gp, P-Glycoprotein

PK, Pharmacokinetic

PL, Phospholipid

PLS, Partial Least Square

PSA, Polar Surface Area

PSE, Process Systems Enterprise

RD, Recommended Dose

RMSE, Root Mean Square Error

rpm, rotation per minute

SD, Starting Dose or Standard Deviation

SGF, Simulated Gastric Fluid

SIF, Simulated Intestinal Fluid

SIVA, Simcyp In vitro Analysis

SLAD, Solubility Limited Absorbable Dose

SLS, Sodium Lauryl Sulfate

$T_{1/2}$, Half-Life

Tm, Melting Temperature

T_{max}, Time to maximum concentration

UGT, UDP Glucuronyltransferase

UHT, Ultra High Temperature

USP, United State Pharmacopoeia

V_{max}, Maximum rate of concentration

Acknowledgments

They say “People make Glasgow” well they are right. All the great people I met during this journey contributed to this unforgettable experience in Scotland.

Three years ago I decided to move to this country I knew almost nothing about. I was just a French guy with a pharmacy degree and I was engaged to the most beautiful and intelligent woman who later became my wife. Unfortunately she could not move with me for many reasons so I want to express how much I love her and how much I missed her during this difficult period of separation. I also want to take this opportunity to thank my parents for always pushing me to study as well as Murielle and Serge (my in laws) for their love and support.

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Declaration of authenticity and author's rights

I, hereby declare that, except where specifically indicated, all the work presented in this thesis is my own and I am the sole author of all parts.

Date:

Signed:

Abstract

During the pharmaceutical development process of a new drug it is necessary to fully elucidate the physico-chemical properties of the molecule in order to find the appropriate formulation. For drugs intended to be administered orally water-solubility is a an essential requirement. The development of high-throughput screening and combinatorial chemistry gave a rapid access to a lot of potential drug candidates but as a consequence led to new challenges. On one hand there is an increasing number of molecules with poor water solubility which can be addressed with adequate in vitro testing. On the other hand studies demonstrated that poor pharmacokinetic behaviour was responsible for the majority of drug candidate failures. Therefore the challenge is to be able to keep up with the rate of production to study the in vivo behaviour of so many drugs more rapidly and efficiently. The emergence of physiologically based pharmacokinetic modelling provides a potential solution by making use of the physico-chemical information along with the human physiology to simulate the clinical performance.

In this work an experimental approach is presented to study the in vitro solubility of poorly soluble drugs. A new biorelevant medium simulating the fasted and fed intestinal states was developed to examine the feasibility of merging the individual fasted and fed studies into one single experiment. The purpose was to forecast the parameters influencing solubility and anticipate the solubility behaviour such as potential significant food effects. Results showed that acidic drugs were greatly driven by pH and oleate while for neutral and basic drugs a combination of three factors (pH, bile salt and sodium oleate) and their interactions were dominant. Solubility testing in biorelevant media has been accepted as a reliable predictive tool of in vivo solubility. The prediction of better estimates of in vivo behaviour allowed BCS class II drugs to be reconsidered provided that an adequate formulation is designed. This is applied in the recent developability classification system (DCS). This classification incorporates an estimate of the fasted intestinal solubility along with the dose/solubility ratio of the drug and allows a better understanding of the factors limiting oral absorption. This classification adds a distinction between dissolution-rate limited drugs and solubility-limited drugs which provides a very useful tool for formulators in the early stage of development. This approach is presented in this work on a set of BCS class II drugs and the consequences on the drug formulation strategy is discussed.

The integration of computational techniques in drug development is getting more and more attention with the immense progress in computational power and the pressure to improve the efficiency and reduce the overall cost of the development process. In this work the use of computer models was employed in two aspects. First, the performance of two simulations software was compared in the ability to predict the solubility of poorly soluble drugs in a fasted

or fed biorelevant medium. Secondly a physiologically based pharmacokinetic (PBPK) modelling method was utilised to simulate the pharmacokinetic profiles of the same drugs. Interestingly both models produced satisfactory results for the solubility prediction of acidic drug and in the fed state however the solubility prediction of the neutral drug and in the fasted state showed some limitations. This outcome highlighted the need for improvement of *in silico* models for solubility prediction in particularly with the development of more sophisticated models for the integration of the multiple components present in the human intestinal fluid. Nonetheless, the successful PBPK prediction of the plasma concentration profiles confirmed the interest of this approach to be a useful tool in the drug development process. In addition the results demonstrated that for two drugs variable input of solubility had a significant influence on the *in vivo* plasma concentrations. Finally a PBPK approach in a special population is presented in this work. It was used to model the pharmacokinetic behaviour of a cancer molecule in patients. The objective was to reproduce the phase I dose escalation study. Due to the sparse *in vitro* data and the poor solubility and permeability profile of the drug, the model development was very challenging and could not be fully validated. In fact the main obstacle of the model was the suboptimal formulation and the absorption of the drug in the intestinal tract. The performance of the software to successfully predict this drug could be challenged by another PBPK software for comparison. However given the physico-chemical properties of this drug its behaviour is very likely to be drug related. This case study emphasised the difficulty of developing poorly soluble drugs.

“Essentially, all models are wrong, but some are useful”

George E.P. Box

Chapter 1
General introduction

1. General introduction

Solubility and dissolution are essential parameters in the absorption process of orally administered solid dosage forms and especially for poorly soluble drugs. According to the classification system introduced by Amidon et al. (Amidon et al. 1995) which categorises the drugs by their high or low solubility and permeability, poorly soluble drugs belong to the BCS class II and IV. This classification system states that absorption of drugs administered as immediate release (IR) dosage forms is determined by three factors which are solubility, permeability and dissolution of the solid dosage form. Typically, after oral intake of a solid dosage form (*e.g.* tablet, capsule) the process of absorption involves disintegration of the formulation, dissolution of the drug substance, gastrointestinal degradation, crossing the intestinal membrane (active or passive transport) and a potential first-pass metabolism in the epithelium and liver before penetrating the general circulation (Figure 1).

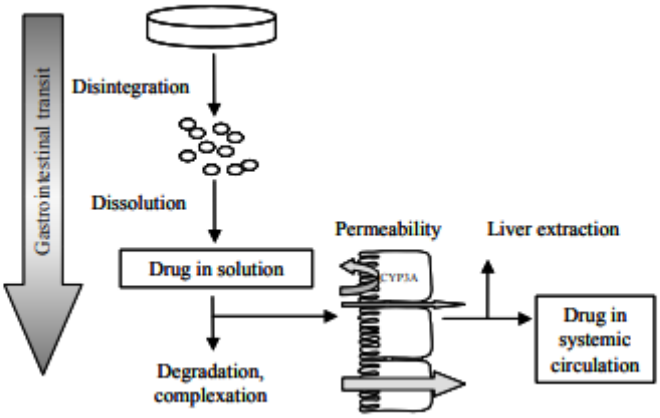

Figure 1 - Schematic overview of drug absorption and bioavailability from a solid dosage form after oral administration

Because a drug must be in solution to be absorbed regardless of the route of administration aqueous solubility is a key factor governing oral bioavailability and as a consequence the pharmacodynamic response. In fact, drugs with insufficient water solubility are associated with inconsistent pharmacokinetic (PK) behaviour and larger inter and intra-individual variability leading to higher risk of failure in the course of the development. A focus is made on poorly water soluble drugs as they can represent up to 70% of new chemical entities (NCEs) (Lindenberg et al. 2004). High throughput screening extensively developed in the nineties contributed to the development of molecules with low aqueous solubility and higher molecular weight (Lipinski, 2002). During the development process solubility testing was only applied in simple aqueous buffers (Alsenz & Kansy 2007) but it is now recognised that the use of biorelevant media reproducing the human gastrointestinal fluid are essential in order to

better predict the *in vivo* behaviour. Therefore physiologically relevant media together with suitable equipment and instrument parameters are of great importance. Since the introduction of the first medium simulating the intestinal fluid two decades ago by Dressman and colleagues (Galia et al. 1998) various biorelevant media have been developed (Bou-Chacra et al. 2017). These media intend to simulate the gastric and intestinal fluids in the two different pre- and post-prandial states based on the available literature data of the detailed composition of human gastric and intestinal fluids. It has been reported that the solubilising potential of the gastrointestinal environment can improve the solubility of low water soluble drugs and as a consequence their bioavailability (Sunesen, Vedelsdal, et al. 2005). Indeed low aqueous solubility does not automatically mean poor intraluminal solubility. It has been demonstrated that intraluminal components can enhance solubility of low water soluble drugs. For example, studies stated that intraluminal mixtures of bile salts increased the solubility of steroid formulations (Bakatselou et al. 1991; Wiedmann et al. 2002). Furthermore the coaction of the phospholipid lecithin with bile salts yielded an even greater positive effect on the solubility of steroids (Naylor, Bakatselou, & Dressman, 1993; B. L. Pedersen, Müllertz, Brøndsted, & Kristensen, 2000.; Wiedmann et al., 2002). After a food intake the concentration of intestinal components is augmented thus the solubility of drugs will be further increased with the release of lipid degradation products from the food content (Clarysse et al. 2009). The formation of mixed micelles by the aggregation of bile salts, lecithin and other lipid products (e.g. monoglycerides) also showed interesting solubilising properties. For instance, the synergy of action of monoglyceride with bile salt was proved to be more efficient for the solubilisation of alpha-tocopherol in comparison to bile salt alone (Nielsen et al. 2001).

During drug development the use of media simulating the intestinal biological conditions aims to anticipate the expected behaviour in human. Because the solubility and dissolution tests are conducted *in vitro,* research scientists seek to link the *in vitro* testing with the *in vivo* outcomes by establishing a reliable *in vitro in vivo* correlation (IVIVC). Therefore the media used must closely mimic the “real life” conditions. The *in vitro* results are then connected to the *in vivo* data (typically the drug plasma concentration profile) and optimisation of development is likely to be achievable. In addition computer models are nowadays able to perform *in vivo* predictions with *in vitro* data. These models are called physiologically based pharmacokinetic (PBPK) models and were built on the extensive knowledge of animal and human physiology and the description of their physiological processes (Jones et al. 2009). The pharmaceutical industry is now pushing its effort toward this approach as it allows reduction of time and cost on animal and human trials.

In this study the solubility of different poorly soluble drugs was studied in a newly developed simulated intestinal fluid using a statistical design of experiment method. This allowed the

determination of the factors influencing the intestinal solubility of poorly soluble drugs. In addition a physiologically based pharmacokinetic approach was applied to study the influence of limited solubility on their *in vivo* behaviour.

1.1. Solubility

In general solubility is defined as the quantity of a substance that can be dissolved in a defined volume of solvent at a given temperature. More precisely solubility can be defined either as equilibrium solubility or kinetic solubility. Equilibrium solubility is defined by the maximum amount of a substance dissolved at equilibrium (usually after 24hrs) under a given temperature and pH. It is also referred to as the thermodynamic solubility and reported as Log S for the logarithmic value of solubility. Equilibrium means that the solution is saturated and the solid form of the substance in contact with the solution is at its most stable form. However in some circumstances related to the substance (*e.g.* crystal form, pKa) or to the solvent (*e.g.* a change in temperature, pressure or pH) the solution becomes supersaturated which means it contains more dissolved substance than the solvent can hold under equilibrium conditions. At this point the solution reaches an unstable state (metastable) meaning the excess substance in solution can crystallise and/or precipitate. The process of crystallisation is initiated by nucleation because it is caused by a nucleus or "seed" which is a minor disruption which will force crystallisation. Kinetic solubility represents any concentration value when the system substance/solute is unstable or not at equilibrium. Kinetic solubility is very convenient in the discovery stages since it requires a relative small amount of compound while thermodynamic solubility is reserved for the later stages of development and intends to help formulation strategies (Alsenz & Kansy 2007).

For ionisable substances the equilibrium solubility can be intrinsic or apparent depending on the ionisation state. Intrinsic solubility (S_o) is the equilibrium solubility at a specific pH where the molecule is fully non-ionised whilst apparent solubility is the equilibrium solubility where the ionised form is also present.

The majority of the drug substances on the market are ionisable (Comer, 2004). They are predominantly made up of molecules with a single acidic or basic group as well as ampholytes. An ampholyte is a molecule with at least two pKa values where at least one of which is acidic and at least one is basic. In the case of a diprotic ampholyte the pKa of the basic group is lower than the acidic pKa so at pH values between the two pKa the molecule is neutral. In the case where the acidic pKa is lower than the basic pKa the molecule is always charged and is called a zwitterion. When ionisable drugs are placed in solution they will dissociate according to the pH of the solution therefore it is important to understand the pH-dependent

solubility of these substances. The pH-solubility profile of ionisable drug substances is calculated using the Henderson-Hasselbalch equation as follow:

Base:	$S = S_0 (1+10^{pKb - pH})$	Equation 1
Acid:	$S = S_0 (1 + 10^{pH - pKa})$	Equation 2
Ampholyte:	$S = S_0 (1+10^{pH - pKa} + 10^{pKa - pH})$	Equation 3

where S is the apparent solubility, S_0 is the intrinsic solubility and pK_a is the dissociation constant. According to the acidic or basic properties of the compounds the solubility will increase or decrease with pH (Figure 2).

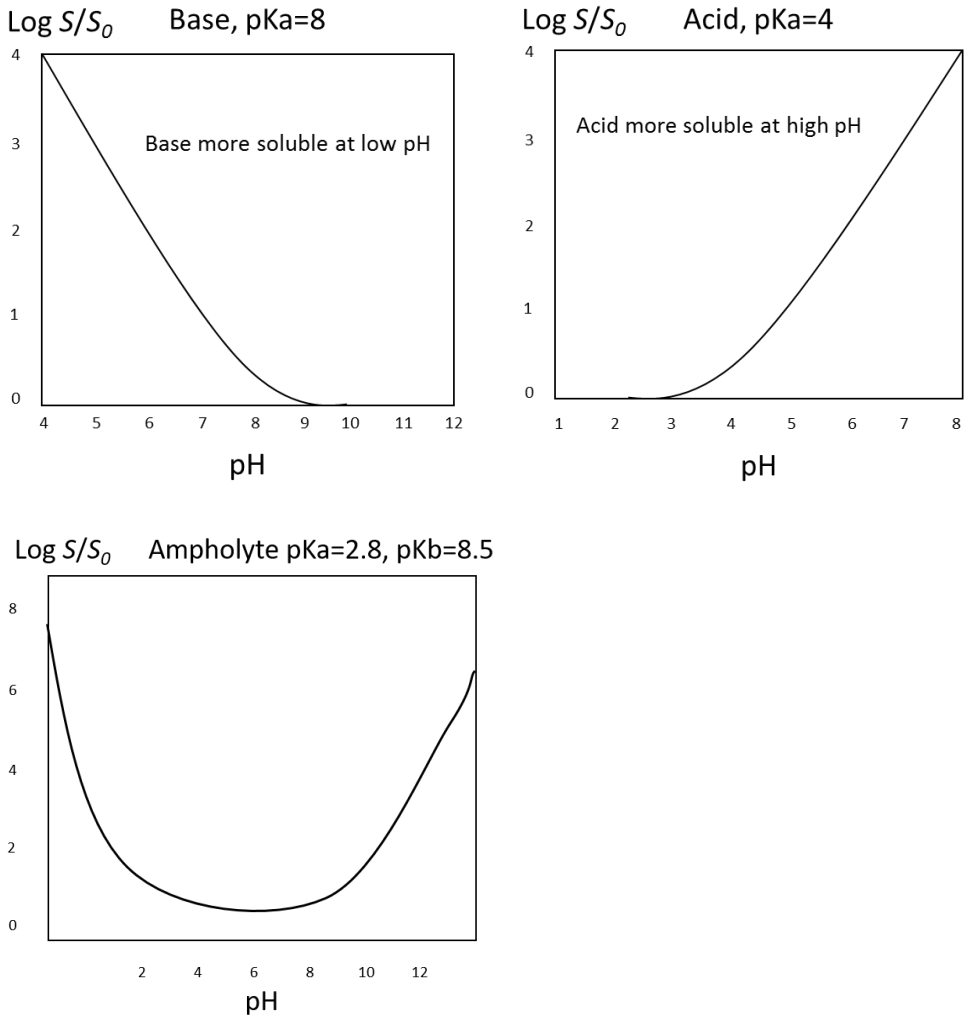

Figure 2 - pH-Solubility profile of ionisable compounds using the Henderson-Hasselbalch equation

Drug solubility also depends on three fundamental parameters. The solvent, which is usually a liquid (single substance or a mixture), temperature and pressure. The term insoluble is very

often used to describe a drug but most insoluble drugs are in reality very slightly soluble. According to the USP 2007 and BP (British Pharmacopoeia 2009) solubility criteria there are seven classes of solubility from “very soluble” to “practically insoluble” (Table 1). When the substance is put in contact with the solvent a dynamic equilibrium is attained and is defined by an equilibrium constant. It is called the solubility product constant (K_{sp}). The formation rate of the dynamic equilibrium can be exceeded in some cases and produce a supersaturated solution where an excessive amount of drug is being dissolved. Supersaturated solutions are very unstable and can lead to crystallisation as mentioned in section 1.1.

Table 1 Solubility criteria according to the United States Pharmacopoeia (USP) and British Pharmacopoeia (BP)

Descriptive term	**Part of solvent required per part of solute**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	10,000 and over

1.2. Methods to measure solubility

The saturation shake-flask method is the classical reference method to measure equilibrium solubility of solid drug substances. It is a simple and reliable approach for neutral and ionisable drug substances but only conducted at a single pH value. However the solubility-pH profile of a drug substance can be performed using several pH buffers. The concentration of the drug substance after the incubation period is usually determined by HPLC with UV detection[1]. Although the shake-flask procedure is simple it is relatively time-consuming and

[1] High-Performance Liquid Chromatography with Ultra-Violet detection

reported studies show important discrepancies in the experimental conditions used. A comprehensive standard protocol was therefore proposed and validated with five test drugs (Baka et al. 2008).

Other techniques available are the potentiometric acid/base titrations which were first introduced by Avdeef and applied to solubility determination (Avdeef 1998). The potentiometric titration principle is based on the precipitation of the drug substance due to variations of pH and only applicable to ionisable drug substances. Two techniques are currently available, the pSol® (pH-metric solubility) and the CheqSol® (chasing equilibrium solubility). In both techniques the drug substance is dissolved at the beginning of the experiment and precipitation is generated by variation of pH. Controlled amounts of acid or base are added to the medium containing the drug substance and the titration curve represents the plot of pH as a function of volume of titrant used. Around the drug's pKa an inflexion of the curve will occur.

The pSol® method is relatively slow and therefore can be inconvenient (8 to 24h per measurement), but on the other hand a single measurement will produce the whole pH/solubility profile with a very small amount of drug substance, (hundreds of micrograms). In addition it has been reported that the data generated are not significantly different from the shake-flask method (Avdeef et al. 2000). An example of use of this method demonstrated its ability to measure intrinsic solubility of polymorphic compounds (Fioritto et al. 2007).

The CheqSol® technique was further developed and described by Stuart (Stuart & Box 2005). It is a simplified and time-effective version of the previous pSol®. This technique allows users to carry out solubility measurement in less than 80 minutes although the pH/solubility profiles have to be further calculated by applying the Henderson-Hasselbalch equation. For example the determination of solubility increases of four test drugs in the presence of excipients demonstrated comparable results with previous literature (Etherson et al. 2014).

1.3. Definition of permeability

Permeability is the capacity of a molecule to go through cell membranes. It is dependent on the properties of the molecule as well as the properties of the membranes and represents one of the essential parameters in drug absorption since it will affect the pharmacokinetic (PK) processes of oral drugs. Permeability is usually reported as effective permeability (P_{eff}) and commonly expressed as a diffusion rate across the membrane (unit cm/s). There are two types of routes for drugs to permeate through the physiological cell membranes (*e.g.* epithelium), the transcellular and the paracellular route.

1.3.1. Transcellular route

The transcellular route is generally the most important for drug absorption. Drug crossing via this route is typically a two step process beginning with the drug uptake from the lumen into the cell (apical side) and ending with drug exiting the cell on the basolateral side (Figure 3). This process can be either passive (passive or facilitated diffusion) or active (carrier-mediated).

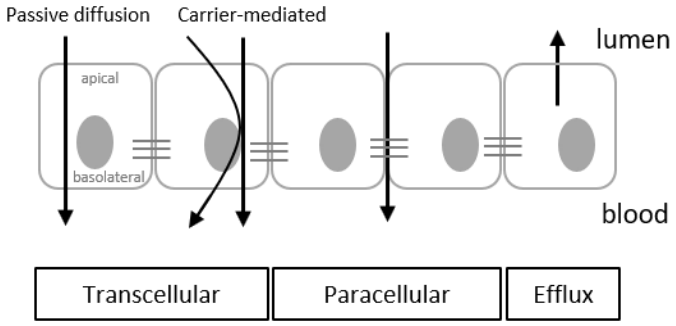

Figure 3 - Schematic representation of the types of route to cross the intestinal membrane. Transcellular which can be active or passive and paracellular through the tight junctions

1.3.1.1. Passive diffusion

Passive diffusion is controlled by a concentration gradient. Drugs will cross from a high concentration region (e.g. lumen) to a low concentration region (*e.g.* blood) with a diffusion rate proportional to the gradient. The rate is also influenced by the size, degree of ionisation and lipophilicity of the molecule therefore diffusion rate will be higher for low molecular weight and lipid-soluble drugs. Lipophilicity (LogP) is a very important parameter in drug discovery. The term literally means “love of fat” and can be defined as the ratio of the non-ionised concentration of a drug between an organic and an aqueous phase. LogP of a given drug defines its affinity for a lipophilic environment. If a molecule has a high lipophilicity value it will be less soluble in a hydrophilic environement. The majority of drugs are ionisable weak organic acids or bases which leads to the definition of Log*D* which considers the ionisation state of the drug and is defined by the lipophilicity at a specific pH. The non-ionised form is generally more lipophilic and easily crosses the cell membrane while the ionised form is more hydrophilic and has limited penetration. The balance between the two forms is determined by the drug’s pKa and the physiological pH. Assuming a monoprotic acid simply expressed as a species HA which dissociates into an anionic A- and a proton H+ at a pH above its pKa then the dissociation of the species can be defined by the following equation when in solution (equation 4). Similarly a monoprotic base can be expressed as a species B which accepts a proton to form a cationic species BH+ below its pK_a value (equation 5). The acid dissociation

constant (K_a) is given by the equation 6 and after taking the log of the Ka (equation 7) and rearranging the equation the Henderson-Hasselbalch relationship (section 1.1) is obtained (equation 8).

Acid dissociation. $HA + H2O \rightleftharpoons A^- + H3O^+$ Equation 4

Base dissociation. $B + H2O \rightleftharpoons BH^+ + OH^-$ Equation 5

$K_a = \frac{[H30+]\,[A-]}{[HA]}$ Equation 6

$\text{Log } K_a = \log [H3O+] + \log\frac{[A-]}{[HA]}$ Equation 7

$\text{pH} = \text{pKa} + \log\frac{[A-]}{[HA]}$ Equation 8

For weak acids when pH is lower than the pK_a the non-ionised form will predominate but for weak bases the ionised form will be dominant. Theoretically, when a drug is given orally weak acids should be more permeable in the stomach (pH around 1.4) while weak bases should be more permeable in the intestine (pH around 6.8) (Figure 4).

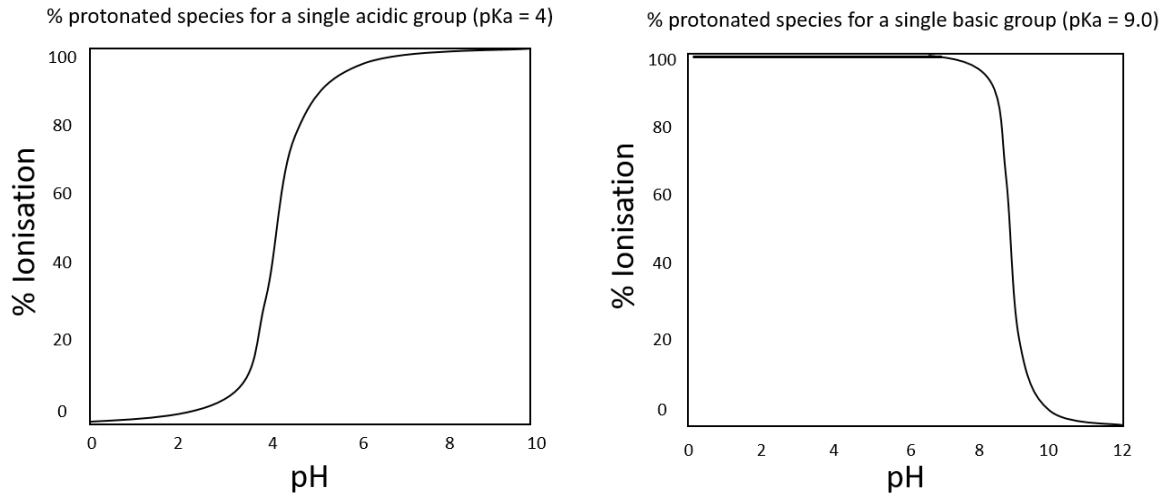

Figure 4 - pH-ionisation profile of an acidic drug with a single pKa and a basic drug with a single pKa

1.3.1.2. Facilitated diffusion

Facilitated passive diffusion is observed with specific molecules characterised by a small molecular weight and a very low lipophilicity (e.g. glucose). A carrier-substrate complex is formed outside the cell and diffuses quickly through the membrane to release the substrate inside. This process is not energy dependent and can only occur with the concentration gradient flow (Sim 2015)

1.3.1.3. Carrier mediated

For carrier mediated diffusion the process occurs according to a concentration gradient. A carrier molecule present in the membrane binds with the substance and the complex travels easily through the membrane releasing the substance inside the cell. The process is relatively selective and limited by the carrier's quantity. The active transport occurs against the concentration gradient and is energy dependent. The process is very selective to specific drug structure comparable to endogenous molecules (e.g. vitamins, sugars, amino acids) and usually happens at specific regions of the small intestine (Sim 2015)

1.3.2. Paracellular route

The paracellular process is the second route of permeation where the drug simply passes between the cells crossing through the tight junctions (Figure 3). Note that both transport processes (transcellular and paracellular) can happen simultaneously for a given drug (Sim 2015).

1.3.3. Efflux

The epithelial membrane also expresses transporters which play a very important role in the permeability and oral bioavailability of drugs. A various number of these transporters such as the *P*-glycoprotein (P-gp), the multi-drug resistance-associated proteins (MRPs) and the breast cancer resistance protein (BCRP) are responsible for the efflux of drugs outside the cell (Figure 3) as their primary objective is to protect the body from harmful substances (Shugarts & Benet 2009).

In order to fully characterise the drugs during the discovery process it is important to study and understand their permeability as most of the time a drug substance will behave very well when tested *in vitro* but will fail to meet the requirements when applied to *in vivo* assays. Therefore a variety of assays presented in the following section have been developed to model drug permeation in the gastrointestinal tract (GIT) and are divided in three categories, *in situ* perfusion model, *ex vivo* diffusion model and *in vitro* model like the Caco-2 model (Volpe 2010). The assessment of permeability is a requirement for the classification in the Biopharmaceutics Classification System (BCS) which will be further discussed in section 1.7. Based on this classification the United-States Food and Drug Administration (US-FDA) grants a biowaiver of human bioequivalence studies for highly soluble and highly permeable drugs (FDA & CDER 2015; Yu et al. 2002). To demonstrate high permeability of a drug the guidance

requires the use of pharmacokinetic studies (absolute bioavailability or mass balance study) but also permits the use of *in vivo* intestinal studies in humans and intestinal permeability models presented herein with the exception of the PAMPA assay and the *ex vivo* models.

1.3.4. In situ perfusion

The *in situ* perfusion is performed on an anesthetised animal (*e.g.* rat) so the integrity of the intestine is maintained (Lozoya-Agullo et al. 2015). A drug in solution is perfused through a cannulated section of the intestine and permeability (P_{eff}) is calculated by the concentration difference between the solute entering and leaving the cannula. This technique has shown a good correlation with *in vivo* perfusion values in humans for drugs permeating via passive diffusion (Fagerholm et al. 1996). However, for carrier-mediated drugs scaling factors were required to fit the data.

1.3.5. Ex vivo models

In the *ex vivo* diffusion method a flat epithelial layer excised from an animal or human is placed between a donor and a receiver chamber (Luo et al. 2013). Drug is added as a solution in the donor side and apparent permeability (P_{app}) is quantified in the receiver side. Measurement can be performed in the absorption direction (lumen to blood) as well as in the efflux direction (blood to lumen). Another *ex vivo* method is available namely the "*ex vivo* Gut sacs" where a part of the intestine is removed from an animal, reversed on a tube and closed at each side to form a "sac" (Alam et al. 2011). It is then placed in a buffer containing the drug. Permeability (P_{app}) is calculated from the quantity of drug in the sac after a period of time. An alternative to this method is to form the sac with the drug solution inside but without reversing the intestine. The amount of drug permeating outside the sac is measured in a buffer solution container. This latter technique was applied to rat intestine sacs and the permeability of 11 commercialised drugs was compared to their respective absorbed human fraction. A good correlation was found with permeability values ranked from 1 to 15x 10^{-6} cm/s (Ruan et al. 2006).

1.3.6. In vitro cell models

The Caco-2 cell model has been extensively used to study permeation of drug candidates since its introduction in 1989 (Hidalgo et al. 1989). The Caco-2 cell line has the capacity to model the intestinal epithelium by forming tight junctions between cells when grown on a semi-porous filter membrane (van Breemen & Li 2005). When placed inside a two-sided chamber,

the permeation of drugs can be studied. Commonly, the test drug is added on one side of the chamber (apical or basolateral) and will follow a concentration gradient or be actively driven by a transporter (Figure 5). Most of the transporter and efflux proteins are expressed along with metabolic enzymes in this monolayer cell model. The apparent permeability (P_{app}) is determined by the quantity of drug received on the other side using equation 9 where the flow rate of the drug appearing over time is noted as dQ/dt, S is the surface area of the cell layer and A_0 is the concentration in the donor side.

Similarly, the MDCK (Madin-Darby canine kidney) cell lines can be used as a surrogate to *in vivo* study to assess the permeability and absorption of drug candidates (Irvine et al. 1999). When seeded on a semi-porous membrane filter it will mimic the intestinal epithelium. The method principle is comparable to the Caco-2 model and P_{app} is calculated using the same equation 9 (Volpe 2008).

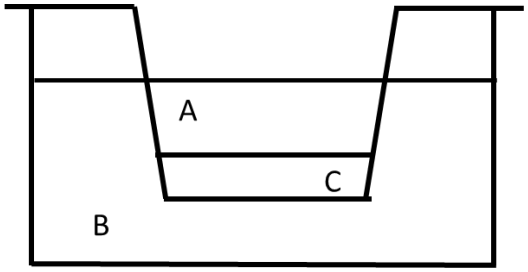

Figure 5 - In vitro cell model (Caco-2 or MDCK), two chambers, apical (A) and basolateral (B) separated by the monolayer cells barrier (C)

$$\mathrm{Papp} = \frac{\mathrm{dQ}/\mathrm{dt}}{\mathrm{S} \times \mathrm{A0}} \qquad \text{Equation 9}$$

1.3.7. In vitro artificial membranes

The interest for the parallel artificial membrane permeability assay (PAMPA) has significantly grown in the pharmaceutical industry since it is a cost effective and rapid test to screen passive-transport of small molecules (Kansy et al. 1998). The method is simple and is conducted on a 96-well plate format. Two aqueous buffer compartments are separated by an organic solvent containing a phospholipid layer which will act as the filtering membrane. It is a non-cellular assay and the study is performed by adding the drug in the donor compartment and measuring appearance over time in the other compartment. Because of the very simplistic model it is usually a complement to a cellular model such as Caco-2 or MDCK models. Nonetheless, successful prediction studies of *in vivo* permeability data have been reported using this technique (Bermejo et al. 2004).

1.4. Absorption and bioavailability

Drug absorption and bioavailability (BA) are often used indistinctly but it is worth specifying that absorption is related to the movement of a drug from a specific site (*e.g.* stomach or intestine) to a blood stream while bioavailability is defined by the rate and extent to which an administered dose reaches the systemic circulation. Absorption is part of the bioavailability process. A drug can have a good oral absorption because of a favourable lipophilicity and yet exhibit a poor oral bioavailability because of an extensive first-pass metabolism (Walle et al. 2004). Oral bioavailability is generally lower than 1 (BA<1) because of incomplete absorption and/or first-pass metabolism as opposed to the intravenous bioavailability which is by definition equal to 1 (BA=1). Different factors can influence the oral bioavailability and they can be related to the drug itself or related to the subject.

The definition of bioavailability given by the FDA specifies that bioavailability "means the rate and extent to which the active ingredient is absorbed from a drug product and becomes available at the site of action" while for the European Medicine Agency (EMA) bioavailability is defined as "the fraction of an administered dose that reaches the systemic circulation" (EMEA 2001; FDA 2002). Defining and particularly measuring a drug at the site of action is difficult therefore the two agencies consider the use of concentrations in the general circulation to determine the bioavailability of oral drugs.

Absorption and bioavailability are fundamental parameters in drug research and development as the administration of a medicine via the oral route is convenient and generally the preferred method (Lipinski et al. 1997; Lipinski 2000). In addition it is the most common approach for the treatment of systemic diseases. As a consequence gastrointestinal absorption plays a decisive role in the therapeutic process (Sim 2015). Following oral administration of a solid dosage form, the drug substance must traverse a list of obstacles (Figure 6) before reaching the systemic circulation. Typically, to reach the systemic circulation a drug must:

a) be released from the solid dosage form
b) be dissolved in the GI fluid
c) avoid metabolism in the intestine
d) permeate across the epithelium barrier (active or passive transport)
e) avoid the metabolism in the epithelium
f) escape efflux from the epithelial cells by transporters
g) avoid metabolism in the portal vein leading to the liver
h) escape the liver metabolism before being released in the systemic circulation

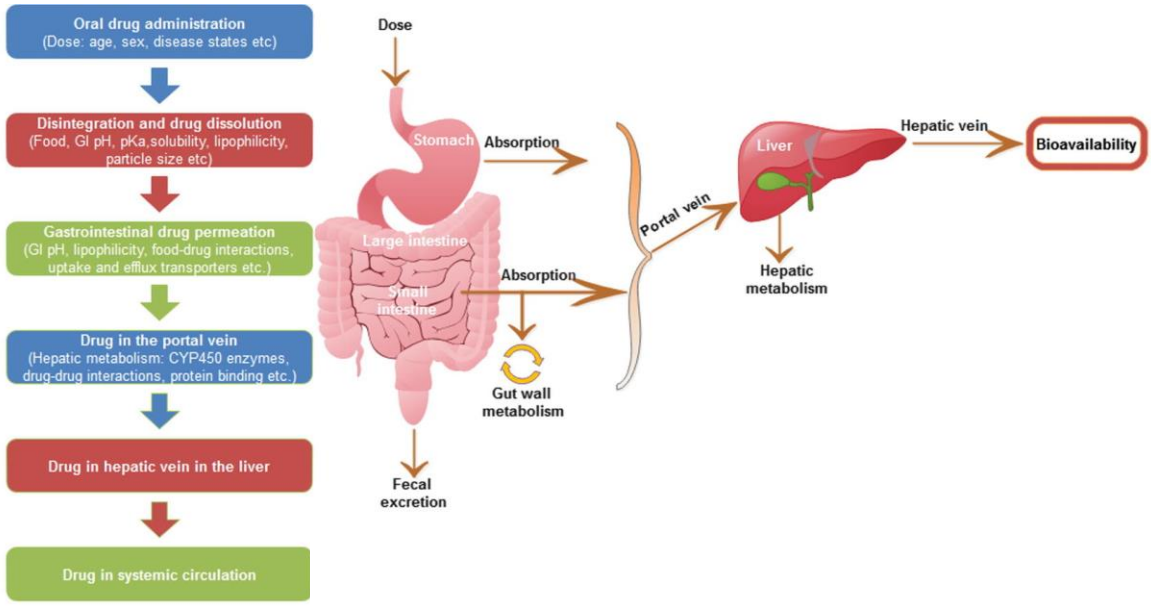

Figure 6 - Typical process of absorption after oral drug administration (Abuhelwa et al. 2017)

The fraction absorbed (F) which is the fraction of the drug that will be available to reach the systemic circulation is determined by the product of the fraction absorbed from the intestinal lumen into the entrocytes (F_{abs}), the fraction escaping the intestinal metabolism (F_g) and the fraction escaping the metabolism in the liver (F_h). It is defined by the following equation:

$$F = F_{abs} * F_g * F_h \qquad \text{Equation 10}$$

Moreover absorption and bioavailability studies are important for the following reasons. Firstly it has been reported that low bioavailability (BA<30%) is associated with high inter and intra-individual variability leading to inconsistent therapeutic response (Beierle et al. 1999; Bardelmeijer et al. 2000; Katsura & Inui 2003). Secondly, a high oral bioavailability reduces the amount necessary to achieve a desired pharmacological effect. Thirdly the number of lipophilic drugs has significantly increased due to modern discovery techniques which result in lower *in vivo* bioavailabilities values from these molecules (Lipinski 2000).
Lipinski and co-workers carried out a comprehensive study on the discovery and development settings of drug candidates regarding solubility and permeability as a barrier to absorption. As a result they established a proposal as a set of five rules (“Rule of 5”) to predict when poor absorption is more likely to occur (Lipinski et al. 1997). According to this rule poor absorption will most likely when the number of hydrogen bond donors is greater than 5, the number of hydrogen bond acceptors is greater than 10, the molecular weight is greater than 500 (daltons) and lipophilicity (Log P) is higher than 5 (Lipinski et al. 1997).

Drug absorption after an oral administration is a complex process and is influenced by many factors such as the physicochemical properties of the drug itself (*e.g.* pK_a, LogP or solubility) as described in section 1.1 and 1.3.1.1 but also by physiological factors (*e.g.* gastric emptying, pH, blood flow, transit time, surface area) and physiopathological conditions (*e.g.* gastrointestinal dysmotility). A brief description of the gastrointestinal anatomy and physiology is presented next to describe the influence of these factors on drug absorption and then the gastrointestinal dissolution of drugs will be introduced.

1.5. Physiology of the gastrointestinal tract

The gastrointestinal tract is a muscular tube of approximately 6 metres long with varying diameters. It spreads from the mouth to the anus and is divided in four main areas namely the oesophagus, the stomach, the small and large intestine. The structure of the wall is essentially the same in all sections of the gastrointestinal tract and consists of four histological layers listed as follows from the outer layer to the inner layer (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001) (Figure 7) .

1. The *serosa* is the outer layer of epithelium composed of several connective tissues with the peritoneum.
2. The muscular layer (*muscularis externa*) is composed of three layers of smooth muscle responsible of the motility and movement of the gastrointestinal content. The outer layer of muscle is thin and longitudinal and the two inner muscle layers are circular.
3. The *submucosa* is composed of an irregular layer of connective tissues densely supplied by blood and lymphatic vessels. It also contains a network of nerve cells known as the submucosa plexus and the enteric nervous plexus.
4. The *mucosa* is the inner layer of the gastrointestinal tract directly in contact with the lumen (*i.e.* inside space of the tubular structure). It is composed of three layers, an outer muscular layer (*muscularis mucosa*) made of a thin layer of muscle, a layer of connective tissue (*lamina propria*) and the innermost layer is the epithelium.

The cells composing the mucosa are highly differentiated in each section of the gastrointestinal tract according to the different functions and particularly for the epithelium which is responsible for the majority of the absorptive and secretory functions. Depending on the section the epithelium can be composed of a single layer or multiple layers (stratified). Furthermore the epithelium is covered by a layer of mucus in the majority of the gastrointestinal tract. It is an aqueous based viscoelastic gel physiologically secreted and its function is to provide a protective mechanical barrier (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001)

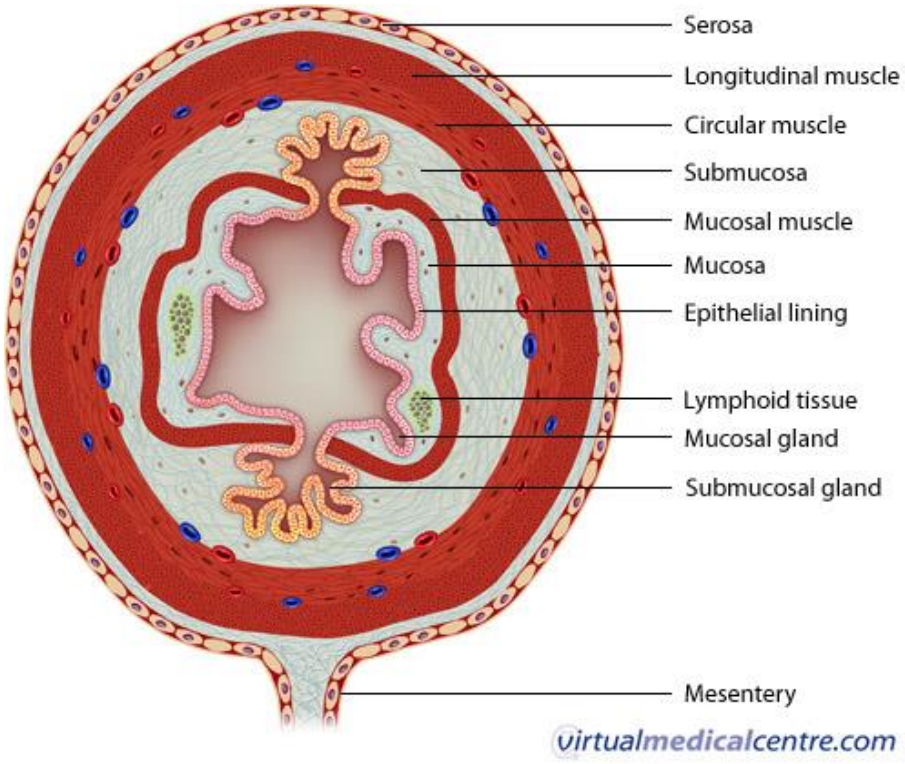

Figure 7 - Cross-section of the gut (Image courtesy of www.myvmc.com)

1.5.1. Oesophagus

The oesophagus is the connection between the mouth and the stomach. It is 25 cm long on average and composed of a heavy muscular layer to move the ingested food down the oesophagus by single peristaltic contrations. The epithelium cells act mainly as a protective layer with the secretion of mucus to lubricate and protect the lower part of the oesophagus from the acidity of the stomach. The pH of the oesophagus varies between 5 and 6 (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001).

1.5.2. Stomach

The stomach is the organ located between the oesophasgus and the small intestine. It consists of four main sections, the cardia next to the oesophagus sphincter, the fundus which is the dilated portion at the top, the body which is the largest section between the fundus and the last section represented by the pylorus. The main functions of the stomach are to act as a reservoir for the ingested food and to control the rate of its delivery to the duodenum by reducing the solids to a uniform consistency with the help of acid and enzyme secretions. The stomach has a volume capacity of approximately 1.5 litres and the pH is between 1 and 3.5 in

the fasted state (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001).

1.5.3. Small intestine

The small intestine is the longest section of the gastrointestinal tract (5 - 6 metres long) and stretches from the pyloric sphincter of the stomach to the ileo- caecal junction where the large intestine begins. It is composed of the duodenum, jejunum and ileum.

The duodenum is the proximal section which receives the content from the stomach and combines the digestive secretions from the pancreas and the liver (bile and enzymes). The jejunum is the following section where a large amount of the digestion and absorption occurs. The last section is the ileum which is the longest part of the small intestine and ends in the caecum. The pH value in the small intestine gradually increases from 6 to 7.4 (Fallingborg 1999). The small intestine is where the majority of absorption of nutrients and drugs happens because of the presence of numerous folds which increases its surface area by around 600 times. Each fold is composed of a number of *villi* (folds of the mucosa) and the surface area of each *villus*, covered by epithelium, is further augmented by *microvilli* (Figure 8).

The wall of the small intestine is full of blood and lymphatic vessels which represents one of the largest systemic regional vasculatures. The blood vessels leaving the small intestine enter the general systemic circulation via the hepatic portal vein and the liver (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001).

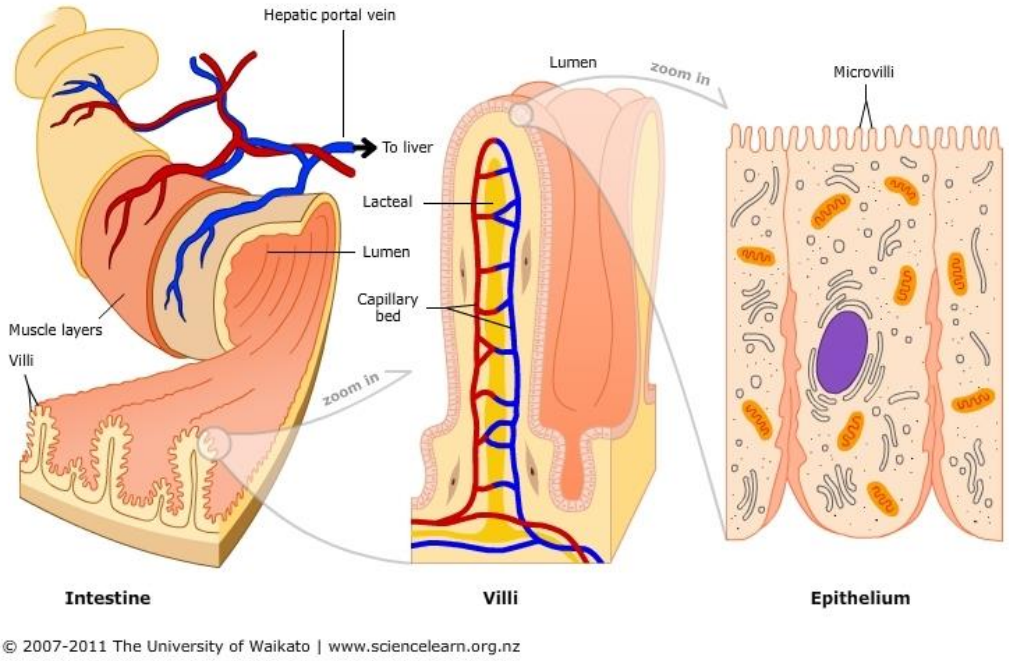

Figure 8 - Villi and microvilli of the small intestine (Science Learning Hub. (2014). Retrieved from www.sciencelearn.org.nz/resources/142-adaptations-of-marineorganisms)

1.5.4. Large intestine

The large intestine is the last section of the gastrointestinal tract. It is around 1.5 metres long and is composed of the caecum, ascending, transverse, descending and sigmoid colon and the rectum. The surface of the large intestine has no villi unlike the small intestine but the epithelial cells still have microvilli. Nevertheless the presence of crypts and sporadic folds in the mucosa increases its surface area by 10 to 15 times. One of the essential functions of the large intestine is to reabsorbe the water from the lumen by the absorption of sodium and chloride ions in exchange for potassium and bicarbonate ions. The other main function is the storage and compression of the faeces. The specificity of the colon is the extensive colonisation by a large variety of bacteria (around 10^{12} per gram of content) responsible for various metabolic reactions such as the hydrolysis of fatty acid esters, the degradation of polysaccharides or the activation of prodrugs in their active form (Martini 5th edition. Fundamentals of Anatomy & Physiology. Prentice Hall. 2001). The pH of the large intestine varies between 5.7 in the caecum to 6.7 in the rectum (Fallingborg 1999).

1.5.5. Factors affecting absorption after oral administration

1.5.5.1. Food effect and splanchnic blood flow

During the fasted state the motility of the stomach is regulated by migrating motor complex (MMC) cycles. Each cycle lasts 2 to 3 hours and is divided in four phases. The ingested liquids can go through freely but solids are transferred from the stomach to the intestine only during the third phase of a motility cycle (Welling 1977). As a consequence gastric residence time of the solid will depend on the time when it is ingested. When food is ingested the pattern of the cycles and the gastric motility are changed. As a result gastric residence time is increased particularly by solid meals, high-fat content and hot meals. A delayed gastric emptying is observed with solid meals while it has been demonstrated that non-nutrient liquid meals may result in the contrary effect and the pH of the stomach is lowered since the presence of food stimulates the secretion of hydrochloric acid (Welling 1977).

When food is transferred from the stomach into the intestine the motility of the intestine is increased and higher secretion of bile and digestive enzymes is also observed. These changes in the gastric and intestine motility are expected to have an impact on the absorption of drugs administered concomitantly. A delayed gastric emptying will delay the absorption of drugs primarily absorbed in the small intestine but not the ones absorbed from the stomach. Acidic drugs will be delayed because of a delay in the transit from the acidic environment of the stomach to the more alkaline environment of the small intestine. Nevertheless drugs with poor solubility in acidic pH could benefit from an increased bioavailability by allowing more drug to be dissolved in the stomach before going in the intestine (Welling 1996).

It has been reported that the ingestion of food may influence the splanchnic blood flow (Dauzat et al. 1994). The splanchnic blood circulation is the network of blood circulations of the splanchic organs organised in parallel to one another. The splanchic organs are the stomach, the small intestine, the colon, the pancreas, the liver and the spleen (Parks & Jacobson 1985). In general the splanchnic blood flow is increased after the ingestion of a solid meal to facilitate the absorption of nutients. In the case of a drug one would expect the absorption to be increased via the splanchnic circulation. However the amount of drug reaching the systemic circulation will depend on the pre-systemic clearance (Melander et al. 1988). A positive food effect (*i.e.* a significant enhancement of bioavailability after the ingestion of food) is commonly associated with a less important pre-systemic circulation, for instance propranolol, propafenone and metoprolol (Liedholm et al. 1990; Axelson et al. 1987; Melander et al. 1977).

1.5.5.2. Intestinal transit time, surface area and lumen concentration

The absorption of orally administered drugs in the gastrointestinal tract is determined not only by the permeability in the gastrointestinal mucosa but also by the surface area, the luminal concentration and the transit rates. The influence of the gastric emptying rate has already been highlighted as an important factor but the transit rate in the intestine also plays a decisive role since it controls the residence time in the larger absorption site. The small intestine has been reported to be more permeable to drug and this is due to the increased surface area along with a reduction of the epithelium in the colon (Masaoka et al. 2006). In fact, most of the drugs are absorbed in the duodenum and jejunum since the presence of villi and microvilli increases significantly the surface area for absorption (Kimura & Higaki 2002). The largest surface areas are represented in the duodenum and jejunum because they possess the highest concentrations of villi and microvilli. On the other hand the smallest surface area is observed in the ileum region (Helander & Fändriks 2014). However some drugs have been reported to exhibit significantly higher permeability in the ileum and colon for instance griseofulvin (Gramatté 1994) and allopurinol (Patel & Kramer 1986) confirming that surface area is not the only parameter determining the region of absorption.

In order to study the influence of luminal drug concentration on the absorption, the water volume available must be known. A volume of 250 ml with solid oral formulations is recommended in clinical studies and is generally accepted to calculate drug concentrations in the lumen since it is difficult to investigate the relation between the effective *in vivo* GI fluid volume and drug absoption (Tanaka et al. 2015). However it must be noted that this volume is affected *in vivo* by gastrointestinal absorption of water fluid and secretions such as bile and pancreatic digestion fluids.

1.5.5.3. Pathophysiological conditions

Gastric and intestinal motility can be increased or decreased because of a therapeutic treatment or a pathological condition. For instance the drug metoclopramide is used in the treamtment of gastroparesis which is a condition observed with diabetic patients where emptying of the stomach does not happen normally (Hasler 2011). The therapeutic effect of metoclopramide will induce an increase in stomach and intestine movements and therefore increase stomach emptying (Lee & Kuo 2010). Similarly the drug cisapride is used to treat patients whose gastrointestinal motility is impaired and therefore reduced or absent. It is indicated in the treatment of adults who suffer from gastroesophageal reflux also known as "heartburn" (Richter & Long 1995). The drug will provoke an increase of the gastrointestinal motility. This category of drugs are prokinetic agents useful in the treatment of motility disorders as they increase the rate of gastric emptying and the upper intestinal motility (Longo & Vernava 1993). However absorption of other concomitant drugs may be affected. The normal rate of absorption of the affected drugs is expected to be increased and expressed by a shorter time to maximum concentration (Tmax) and increased maximum plasma concentration (Cmax) (Greiff & Rowbotham 1994). Other categories of drugs may have an important influence on the gastrointestinal motility such as oral analgesics (opioids) or drugs with anticholinergic or sympathomimetic properties (Leppert 2012). Although the available literature data only report limited clinical impact, it may be sensible to be very careful when dealing with certain drugs such as narrow therapeutic index drugs.

1.6. Gastrointestinal dissolution of drugs

Dissolution is the ability of a drug to go into solution and is conditioned by the aqueous solubility of this drug. After oral administration of a solid dosage form a drug must be released from its formulation and be dissolved before permeating through the gastrointestinal epithelium. Therefore bioavailability is also governed by dissolution rate especially for poorly soluble drugs. When a drug has a limited aqueous solubility it will generally be problematic for dissolution. The ratio of the highest dose (mg) over its aqueous solubility (mg/ml) is a useful indicator for limited dissolution. This ratio represents the volume of fluid in the GI tract which will be needed to dissolve the dose administered. Assuming a volume of 250 ml present in the gut after an oral administration, when this ratio is lower or equal to 250 ml absorption will be complete since the drug dissolution will not be solubility limited (Table 2). When the ratio is between 250 ml and 1000 ml absorption of the drug would certainly be incomplete since the drug dissolution will be solubity limited. Finally if the ratio is greater than 1000 ml then solubility and dissolution of the drug will be an issue for absorption.

Table 2 Effect of the fluid volume on the dissolution and absorption of drugs

Ratio **highest dose / aqueous solubility**	**Absorption**	**Dissolution**
≤ 250 ml	Complete	Not limited
250 < Ratio < 1000 ml	Incomplete	Solubility limited
Ratio > 1000 ml	Incomplete	Problematic

Noyes and Whitney (Noyes & Whitney 1897) were the first to demonstrate a dissolution model of solid substances. This model was later revised by Nernst and Brunner (Nernst. W. 1904; Brunner. E. 1904) by including the diffusion coefficient and diffusion layer thickness (equation 11).

$$\mathrm{DR} = \frac{\mathrm{A} \times \mathrm{D} \times (\mathrm{Cs} - \mathrm{C})}{\mathrm{h}} \qquad \text{Equation 11}$$

Where *DR* is the rate of dissolution, *A* is the available surface area of drug particles, *D* is the diffusion coefficient of the drug, *Cs* is the saturated concentration of the drug, *C* is the concentration of the drug in the dissolution medium (solubility) and *h* is the thickness of the diffusion boundary layer.

According to this equation the rate of dissolution of a solid drug in a medium is proportional to the surface area of drug particles, the diffusion coefficient and the saturated concentration of the drug. In other words the dissolution and therefore the solubilisation of the drug will increase with increases of these parameters. On the contrary, if the height of the diffusion layer (*h*) increases the rate of dissolution and solubilisation will decrease.

1.6.1. Factors affecting the surface area

1.6.1.1. Particle size reduction

The particle size of a drug is indirectly proportional to the surface area as it will increase when the particle size decreases. This “micronisation” technique is a well-known and successful technique to improve dissolution in the development phase of a drug (Chaumeil 1998). The reduction of particle size to micrometre sizes has shown successful results in the enhancement of bioavailability of poorly soluble drugs such as griseofulvin, spironolactone

and progesterone (Chaumeil 1998). In addition it has been demonstrated that nanosizing and nanosuspensions can lead to even greater enhancement of bioavailability compared to micronisation with good examples of marketed drugs belonging to BCS class II and IV administered orally as nanosuspensions (Merisko-Liversidge et al. 2003; Kesisoglou & Mitra 2012).

1.6.1.2. Surface wetting

The wettability properties of the dissolution media fluids are essential to effectively wet the surface area. Wetting capacity is improved by the presence of surfactants in the GI tract particularly for poorly soluble drugs (Bakatselou et al. 1991). Contrastingly with the particle size reduction technique it has also been reported that if the dissolution medium has very poor wetting capacities micronisation can have a negative effect on dissolution rate (Solvang & Finholt 1970).

1.6.2. Factors affecting the diffusion coefficient

The diffusion coefficient (D) is defined by the Stokes-Einstein equation (equation 12) where k is the Boltzmann constant2, T is the absolute temperature, π is equal to 3.14, η is the medium viscosity and r is the particle radius (Edward 1970).

$$D = \frac{k \times T}{6 \pi \eta r} \qquad \text{Equation 12}$$

According to equation 12 viscosity is inversely proportional to diffusion coefficient and therefore inversely proportional to dissolution rate.

The fluid in the gastrointestinal tract can be more or less viscous depending on the food composition and the fluids co-administered. Ingredients such as fibres (*e.g.* pectin, guar) increase viscosity. High concentration of amphiphilic molecules can also increase viscosity and furthermore by formation of micelles. For instance, it has been reported that a hydrocortisone solution with a mixture of sodium taurocholate (NaTC) plus lecithin has a much smaller diffusion coefficient value compared to a solution of sodium taurocholate alone (Naylor et al. 1993).

[2] Boltzmann constant = $1.38064852 \times 10^{-23}\ J.K^{-1}$ (J = Joules, K = Kelvin)

1.6.3. Factors affecting the saturated concentration solubility

Saturated solubility of a drug in the gastrointestinal tract is influenced by physicochemical and physiological factors such as its crystalline form, lipophilicity, pKa, biorelevant surfactant and the pH profile in the GI tract (section 1.1).

1.6.3.1. Crystalline and amorphous form

According to the arrangement of its molecules a solid (*e.g.* a solid drug substance) is classified either as a crystalline or an amorphous solid. In an amorphous solid the constituent molecules are arranged in an irregular manner with no long range structure and therefore they are defined as materials with no organised arrangement of atoms and molecules. On the other hand in a crystalline solid the molecules are arranged and organised in a regular manner with a specific geometry. The advantage of crystalline solids is their stability and solidness compared to amorphous solids however they are less soluble because of the higher energy necessary to break the strongly organised pattern of the crystal. As a consequence most of the drugs exist as amorphous forms because of their higher solubility but the reliable measurement of their solubility with accuracy remains a practical problem (Murdande et al. 2011). A variation of the solubility will have an effect on dissolution rate however it must be noted that an increased dissolution does not always improve the bioavailability (Dresse et al. 1978).

1.6.3.2. Biorelevant surfactants and lipophilicity

The extent of solubilisation of a drug is correlated to its lipophilicity (Attwood & Florence 1983; Barry & El Eini 1976). The presence of amphiphilic bile components in the small intestine can improve the solubility of drugs by forming micelles when their concentration is greater than the critical micelle concentration (CMC). This is usually the case for poorly soluble drugs where solubility of 100 times higher has been found when physiological concentrations of bile salts have been added to an aqueous medium, for instance griseofulvin (Elworthy & Lipscomb 1968). The use of lecithin with bile salt can also improve the solubilisation capacities of mixed micelles by increasing their molecular weight (Shankland 1970). Furthermore the importance of the bile salt to lecithin ratio was reported for diazepam and the solubilisation is greater when the lecithin to bile salt ratio increases (Rosoff & Serajuddin 1980).

1.6.3.3. pKa and pH profile of the GIT

As aforementioned in the solubility section 1.1. solubility of ionisable drugs is correlated to its pKa and the physiological pH along the gastrointestinal tract. For weak acids solubility increases with pH while for weak bases solubility decreases. For neutral drugs (*i.e.* non-ionisable) solubilisation and dissolution will not be influenced by pH modifications in the GI tract. Saturated solubility is therefore widely dependent on the physiological pH of the stomach and intestine.

1.6.4. Factors affecting the diffusion boundary layer thickness

To increase the dissolution rate (*DR*) the diffusion layer thickness (*h*) must be reduced. The thickness of the diffusion layer is influenced by the degree of agitation of the medium surrounding the drug particles. The normal peristaltic movement of the gastrointestinal tract motility may increase the dissolution rate by reducing the thickness of the layer around the drug particles however for solubility limited drugs it will be difficult to improve the normal motility and therefore the dissolution rate.

1.7. The biopharmaceutics classification system (BCS)

The biopharmaceutics drug classification was first introduced by Amidon and co-workers (Amidon et al. 1995). In this paper drug substances are classified according to their aqueous solubility and intestinal permeability in four different classes as follow (Figure 9). Class 1: High solubility - High permeability drugs, Class 2: Low solubility - High permeability drugs, Class 3: High solubility - Low permeability drugs and Class 4: Low solubility - Low permeability drugs. When combined with the dissolution of the drug formulation it is acknowledged that those three factors are controlling the rate and extent of absorption of immediate release solid oral dosage forms. A drug substance is defined as highly permeable when its fraction absorbed (F) is greater or equal to 85% or 90% depending on the regulatory authority (FDA & CDER 2015; Davit et al. 2016) and highly soluble when the dose number (D_0) is less than 1. The dose number parameter is dimensionless and calculated as follows:

$$\mathrm{Do} = \frac{\mathrm{Dose}}{250\ \mathrm{mL\ x\ Cs}} \qquad \text{Equation 13}$$

where the Dose is the maximum dose strength available on the market and *Cs* is the lowest solubility in mg/ml of the drug over the GI tract pH range as discussed in section 1.6.

Class 1. In this case drugs are rapidly dissolved and absorbed. The absorption rate will depend on dissolution or the gastric emptying of the formulation if the drug dissolution is very

rapid. However the systemic availability can be low if the first pass extraction/ metabolism is high.

Class 2. For this class of drugs, the permeability is high thus drug dissolution is the limiting step and therefore it must be well studied. The limited dissolution will expose the drug to the intestinal membrane for a longer period. The studies must be conducted at different time points and pH as the characteristics of the membrane change along the intestine as presented in section 1.5.

Class 3. This is the case where poor permeability is limiting the absorption while solubility is high so the dissolution profile of this class of drug should be comparable to class 1 for immediate release dosage forms. The variations of drug absorption in this class will be caused by luminal contents and membrane specifications (transit time, permeability) rather than dosage forms.

Class 4. Drugs in this class exhibit significant issues for an effective oral delivery. Limited permeability and aqueous solubility lead to a very poor absorption and significant inter and intra-individuals variability. The development of an effective formulation is an immense challenge for this category of drugs.

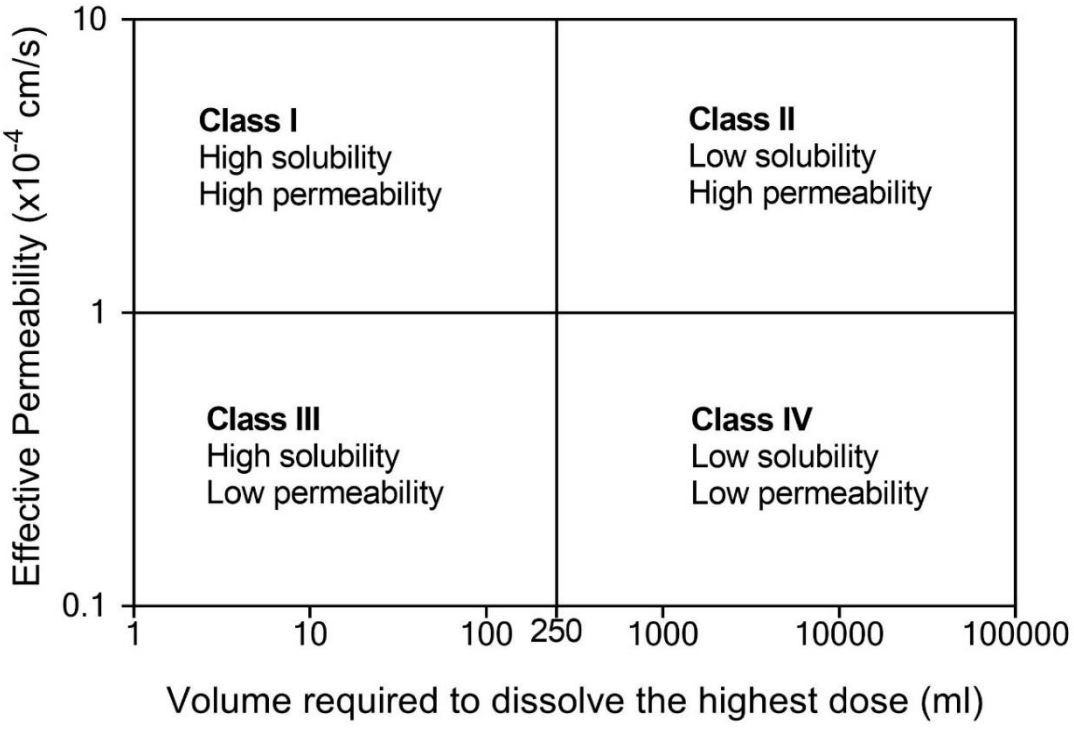

Figure 9 - The biopharmaceutics classification system (BCS) (Amidon et al. 1995).

1.7.1. BCS-based biowaivers and application

BCS-based biowaiver is an exemption of *in vivo* bioavailability and bioequivalence (BA/BE) studies for certain solid oral dosage forms under specific circumstances. Authorisation of

biowaivers by regulatory authorities is a way to avoid unneeded human studies as much as possible and promote easiest access to drugs in developed countries as much as in emerging countries. The regulatory agencies have acknowledged that two immediate release (IR) products should present the same rate and extent of absorption if (i) they act like oral solutions in the GI tract because of their rapid dissolution and high solubility, (ii) their drug substances are not subject to precipitation and (iii) the two formulations display the same *in vivo* dissolution profile along the different intestine conditions. As a consequence the two formulations must be bioequivalent (Davit et al. 2016).

1.7.1.1. Application of BCS based biowaiver

The application of a BCS based biowaiver is relevant in any regulatory scenario where bioequivalence studies are required for an immediate release (IR) solid oral dosage form (Davit et al. 2016). Two formulations are assumed to be bioequivalent if their rate and extent of absorption are not significantly different when given to subjects or patients under the same dosing and experimental conditions. Demonstrating bioequivalence can be required for generic and also innovator products. For the development of generic drug, bioequivalence must be demonstrated between the generic candidate and the reference product in order to be approved. For new drug applications a bioequivalence study must be performed between the formulation used in the final clinical study (phase III) and the clinical scale "to-be-marketed" formulation. In addition a BE study is required for any types of scale-up or post-approval changes (Davit et al. 2016).

1.7.1.2. European Medicine Agency and US Food and Drug Administration

In 2000 the Food and Drugs Administration (FDA)[3] guidance was the first to encourage the use of biowaivers based on the work of Amidon and co-workers followed by the European Medicines Agency (EMA)[4] in 2001 (Davit et al. 2016). According to both guidances waiver of *in vivo* bioequivalence studies could be granted for immediate release solid oral dosage forms that fulfil the *in vitro* qualifications related to solubility, permeability and dissolution. It is assumed that equivalent bioavailability of a drug product will be obtained if solubility and permeability are high along with a rapid dissolution of the formulation. The criteria allowed the consideration of Class 1 drugs only. High permeability is obtained when the extent of

[3] US Food and Drug Administration. Draft guidance for industry, waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system. 2015

[4] European Medicines Agency. Guideline on the investigation of bioequivalence 20 Doc. Ref.: CPMP/EWP/QWP/1401/98 s.l.: Com- mittee for Medicinal Products for Human Use (CHMP). 2001

absorption in humans is greater than 90% of an administered dose. Studies to support permeability can be mass-balance pharmacokinetic studies or intestinal permeability models as mentioned in section 1.3. High solubility is defined as the highest dose strength soluble in less than 250 ml of water over a pH range of 1 to 7.5. Two types of studies can be provided to demonstrate the solubility, pH-solubility profile in aqueous media over the pH range and shake flask method. The volume limit value of 250 ml is taken from a standard clinical study where a glass of water (8 ounces = 240 ml) is administered with an oral formulation. Assuming a residual volume of 10 ml in the GI tract in the fasted state an oral formulation will be exposed to a total volume of 250 ml. The dissolution of a formulation is classified as rapid when more than 85% of the drug substance is dissolved within 30 minutes. Studies to support dissolution test must be performed at three physiological pH values 1.2 - 4.5 and 6.8 using the United States Pharmacopoeia (USP) apparatus I (basket 100 rpm) or USP apparatus II (paddle 50rpm) in a buffer dissolution volume of less than 900ml (Benet 2013).

In 2010 the European Medicines Agency (EMA) published new guidelines considering the extension of BCS-based biowaivers application to class III drugs. Subsequently the FDA revised its guidance and released a new draft in 2015 to also consider class III drugs. The disparities observed during the period between 2000 and 2015 might explain the small number of BCS-biowaiver submissions. Divergences between the two regulatory bodies still remain but the present approaches are in acceptable accordance.

1.8. The Developability Classification System (DCS)

This classification system was proposed by Butler and colleagues (Butler & Dressman 2010) using the previous biopharmaceutics classification system as a frame. The introduction of the BCS had significantly influenced the development of immediate release oral dosage forms by allowing the use of *in vitro* data alone to demonstrate bioequivalence for class I drugs. However, the system remains inflexible regarding the assessment of BCS class II drugs for which absorption is limited by solubility and/or dissolution rate. With the increasing use of more realistic methods to estimate *in vivo* solubility and dissolution, the DCS classification was proposed to potentially include other classes of molecules and particularly BCS II drugs. This revised version of the original BCS is designed to more accurately classify drugs with the use of better estimates of solubility and a more sensible fluid volume in the GI tract.

One theoretical concept behind this new classification is the compensatory effect between solubility and permeability. This is based on the calculation of the maximum absorbable dose (MAD) widely used in drug development as follows (Sun et al. 2004).

$MAD = P_{eff,\ human} * S * A * T_{SI}$ Equation 14

Where *S* is the solubility, *A* is the absorption surface area, *Peff* is the human jejunal permeability, and T_{SI} is the transit time of 3.32h for the small intestine. This equation from Sun et al. (Sun et al. 2004) includes an estimate of the effective human jejunal permeability and suggests that a high permeability can compensate for a low solubility when calculating the maximum dose absorbable in the gastrointestinal tract. Therefore, the objective of this new classification is to consider the parameters limiting oral absorption in order to better classify the drugs. It is intended to assist formulators in product development rather than being a regulatory classification only. The main difference between the two classifications is the assumed volume available for drug dissolution, 500 ml instead of 250 ml for the BCS classification. The following parameters are incorporated (Figure 10).

1) An estimated human intestinal solubility values (*e.g.* FaSSIF)
2) The solubility limited absorbable dose (SLAD)
3) The dissolution rate of the drug

The estimated human intestinal solubility is an experimental study of solubility in any appropriate biorelevant medium simulating the fasted intestinal fluid, for example the fasted simulating intestinal fluid (FaSSIF). The solubility limited absorbable dose represents the dose above which absorption is limited by the solubility. Beyond this point linear dose response may be lost. It is depicted on Figure 10 as the limit between class II-a and II-b and between the class III and IV (blue solid line) and expressed as follow:

$SLAD = S_{si} \times V \times An$ Equation 15

where S_{SI} is the estimated solubility in the intestine, *V* is the intestinal volume of 500 ml and A_n is the absorption number. The absorption number (An) is calculated using equation 16 where P_{eff} is the effective permeability, *R* is the radius of the intestine and t_{res} is the residence time in the intestine.

$$A_n = \frac{Peff}{R} \times t_{res}$$ Equation 16

For class II-a drugs, complete absorption is expected since permeability is high but factors influencing dissolution (*e.g.* surface area, diffusion coefficient and diffusion layer thickness) will be decisive. On the other hand to reach complete absorption the drugs in class II-b will require a solubilised form.

The authors of the DCS state that for dissolution rate limited drugs (class II-a) the dose/solubility ratio is less pertinent therefore a target particle size is suggested below which dissolution will not become problematic. The equation used to calculate the particle size (r^2) is detailed as follow:

$$r^2 = (3D/\mathrm{Dn})(\mathrm{Cs}/\rho)\mathrm{T_{si}} \qquad \text{Equation 17}$$

where *D* is the diffusion coefficient, *Dn* is the dissolution number, *Cs* is the saturated drug concentration ρ is the drug density and T_{si} is the small intestine transit time.

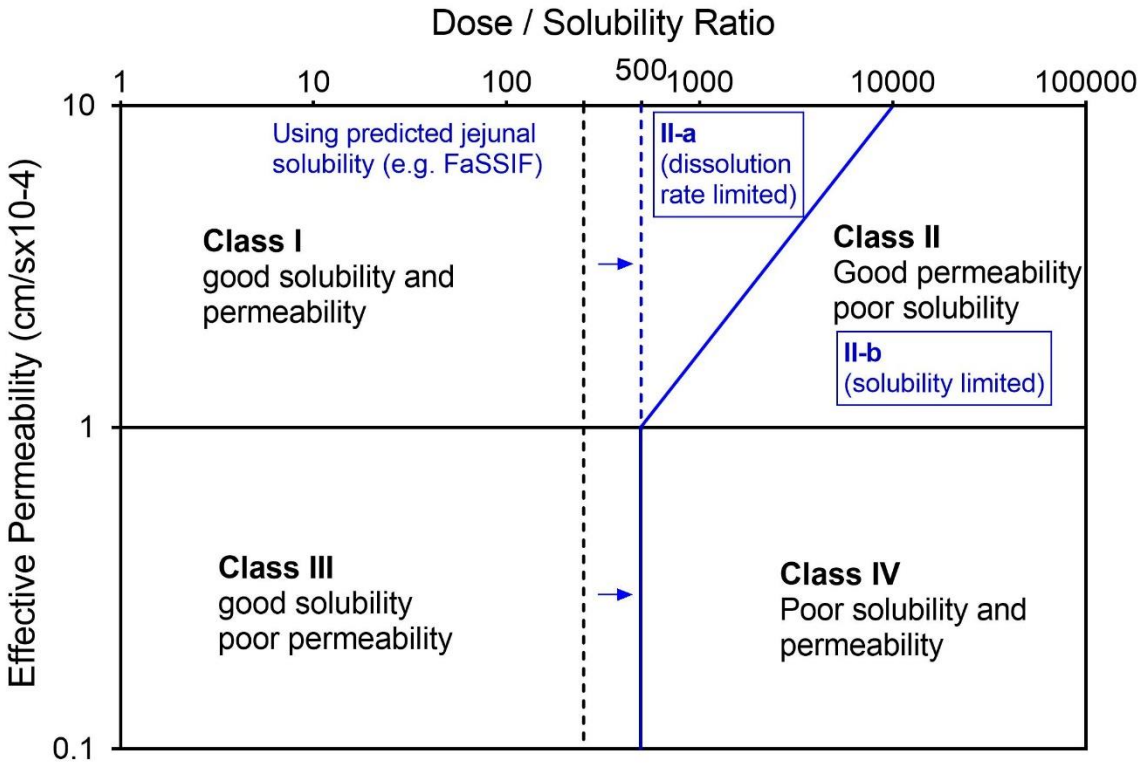

Figure 10 - The BCS/DCS plot with human jejunum permeability (y-axis) and ratio of dose over solubility (x-axis) (Butler & Dressman 2010)

1.9. Human gastro-intestinal fluid characterisation

The traditional media used to simulate gastric and small intestine conditions are the simulated gastric fluid (SGF) and simulated intestinal fluids (SIF) of the United States Pharmacopoeia (USP)[5] standard tests. The compendial SGF has a pH of 1.2 (hydrochloric acid, HCl) and contains sodium choride (NaCl) (34.2mM), sodium lauryl sulfate (SLS 0.25%, w/v) and water

[5] United States Pharmacopeia (USP), U.S. Pharmacopeial Convention, Inc. Rockville, MD

(USP). The simulated intrestinal fluid (SIF) has a pH of 6.8 and contains dihydrogen potassium phosphate (KH_2PO_4), sodium hydroxide (NaOH) and water (USP). Although these compendial media are very useful for quality control, important factors are not considered such as the influence of food. As a consequence, measuring the solubility and dissolution of a drug in an aqueous medium is not sufficient to predict the gastrointestinal environment. Oral absorption and bioavailability can be underestimated especially for poorly soluble and very lipophilic drugs. The simulated media must reflect at best the solubilising characteristics of the human intestinal fluid (HIF) as the use of the latter is limited. This is why fasted and fed simulated gastric fluids (FaSSGF, FeSSGF) along with fasted and fed simulated intestinal fluids (FaSSIF and FeSSIF) have been developed. The first biorelevant medium to simulate the intestinal fluid was proposed by Galia and co-workers in 1998 (Galia et al., 1998.). The main application of this medium was to assess solubility and dissolution of poorly soluble drugs (BCS class II and IV) although it has been extended to other fields such as drug stability, permeability and simulation of gastric emptying. Since then, different studies have tried to modify these simulating media (Boni et al. 2007; Rupp et al. 2010; Söderlind et al. 2010). Updated version of FaSSIF (FaSSIFv2 and v3) and FeSSIF (FeSSIFv2) were published in 2008 and 2015 (Jantratid et al. 2008) (Fuchs et al. 2015).

1.9.1. Simulated gastric fluid

1.9.1.1. Fasted state

The first medium simulating the fasted gastric fluid (FaSSGF) was reported by Vertzoni and colleagues (Vertzoni et al., 2005). It was composed of sodium taurocholate (80µM), lecithin (20µM), pepsin (0.1mg/ml), NaCl (34.2mM) and had a pH of 1.6 (Table 3). In comparison with the SGF of the US pharmacopoeia, pepsin was used instead of the synthetic surfactant sodium lauryl sulfate (SLS). Bile salt (sodium taurocholate, NaTC) was added as well as the phospholipid (PL) lecithin and the pH was changed from 1.2 to the more physiological value of 1.6 (Table 3). A simulated gastric medium without pepsin was also reported but in the study it was used only for the drug ketoconazole (Galia et al. 1998) (Table 3). Further Vertzoni and colleagues determined the solubility of four BCS class II drugs in human gastric aspirates, canine gastric aspirates and in four different simulated gastric fluids including the one published in 2005. The aim of this study was to propose a medium for estimating gastric soubility relevant to a bioavailability study. Although they demonstrated a better basis for the evaluation of intra-gastric solubility during a bioavailability study with the use of simulated gastric fluids the solubility mmeasures were still not accurate enough when compared to the human gastric aspirates (Vertzoni et al. 2007). Furthermore Jantratid and coworkers

published a review to provide an update of the different dissolution media simulating the conditions in the proximal human gastrointestinal tract (Jantratid et al. 2008). In this review a fasted state gastric fluid is suggested (Jantratid et al. 2008). It must be noted that in this study the volume of the simulated gastric medium is 1 litre. This volume does not reflect the gastric content. In the stomach the fluid secretion is usually low but with the ingestion of a glass of water (240 ml) a volume around 250 ml is much more relevant (Klein 2010). Since then no further study describing a simulated medium of the fasted state in the stomach has been reported.

Table 3 Composition of the different fasted simulated gastric media

Composition	**SGF** (USP)	**SGFsp** (Galia et al. 1998)	**FaSSGF** (Vertzoni et al. 2005)
Sodium lauryl sulfate (%, w/v)	0.25	-	-
Triton X100 (%, w/v)	-	0.1	-
Pepsin (mg/ml)	-	-	0.1
NaTC (µM)	-	-	80
Lecithin (µM)	-	-	20
NaCl (mM)	34.2	34.2	34.2
pH	1.2	1.2	1.6

SGF = Simulated gastric fluid without pepsin, SGFsp = Simulated gastric fluid without pepsin, FaSSGF = Fasted state simulated gastric fluid, USP = United states pharmacopoeia, NaTC = Sodium taurocholate, NaCl = sodium chloride

1.9.1.2. Fed state

During the fed state, an important increase of the gastric residence time is observed (Stotzer & Abrahamsson 2000) so dissolution of some immediate release formulations might occur in the stomach. Therefore the time to reach the plasma will very likely depend on the drug dissolution profile. Trying to reproduce the physiological properties of the fed stomach is an

important challenge. Its composition and pH will evolve from the beginning of the digestion through the emptying of the stomach and as mentioned previously it largely depends on the content of the meal. A number of studies have tried to develop fed gastric conditions (Ashby, Beezer, & Buckton, 1989; Buckton, Beezer, Chatham, & Patel, 1989; Junginger, Verhoeven, & Peschier, 1990.). However the media developed do not assess the critical parameters involved in the food effect like the contribution of the meal to the composition of the fluid. Moreover the pH values are not representatives of the conditions after food ingestion. According to a previous study during the fed state the gastric pH declines steadily after food intake from pH 6.4 to 2.7 (Kalantzi et al. 2006). The most suitable medium to assess initial gastric state in the fed condition should possess the nutritional properties of a standardised meal (*e.g.* the breakfast recommended in the guidance for industry of the FDA (Food and Drug Administration (FDA) 2002) and it should be possible to simulate the evolution of the content with time. First the use of milk was considered to mimic the fed conditions in the stomach (Macheras et al. 1986) and Ensure® Plus was introduced many years later (Klein et al. 2004). Their compositions are similar to a standard breakfast meal regarding the ratio carbohydrate / fat / proteins. They also have a pH and physicochemical characteristics comparable to homogenized standard breakfasts.

A single published paper reports a simulated medium for the fed state in the stomach. UHT-Milk was used with buffer in the same proportion to mimic the secretion of gastric juice and pH set to 5 (Jantratid et al. 2008) (Table 4).

Table 4 Composition of the fed simulated gastric medium

Composition	**FeSSGF**
Sodium chloride (mM)	237.02
Acetic acid (mM)	17.12
Sodium acetate (mM)	29.75
Milk/acetate buffer	1:1
pH (HCl / NaOH)	5

FeSSGF = Fed state simulated gastric fluid, HCl = hydrochloric acid, NaOH = Sodium hydroxide

1.9.2. Simulated intestinal fluid

1.9.2.1. Fasted state

The first fasted state simulated intestinal fluid (FaSSIF) medium proposed by Dressman and co-workers was using a monophosphate buffer (KH_2PO_4) which pH of 6.8 described the values observed from the mid-duodenum to the proximal ileum (Dressman et al. 1998). In addition it contained bile salts (sodium taurocholate) and phospholipids (Lecithin) at a concentration of 5 mM and 1.5 mM respectively for a ratio of 3.3 (Table 5). Similarly another version of simulated intestinal fluid was published the same year with different concentrations of bile salt and lecithin resulting in a ration of 1 to 4 and pH was adjusted to 6.5 (Galia et al. 1998) (Table 5). The use of lecithin is interesting because of its important concentration in the bile secretions (Kleberg, J. Jacobsen, et al. 2010). The two major sources of lecithin used in biorelevant media are eggs and soy with egg lecithin showing a higher quantity of saturated fatty acids. A large egg contains 1.5 grams of saturated fat which represents 2.3 percent of the daily intake value in human. In the FaSSIF-V2 the bile salt / phospholipid ratio was raised from 4 to 15 due to a reduced phospholipid concentration (0.2mM) (Jantratid et al. 2008). Moreover the phosphate buffer was replaced by maleic acid to agree more with lower physiological osmolarity. Assuredly the most biorelevant and suitable buffer in the human fasted duodenum and jejunum is bicarbonate as it is physiologically secreted with pancreatic juice (Boni et al. 2007). Unfortunately it has a weak buffer capacity and poor reproducibility. Therefore using maleate buffer was recommended in this updated version (FaSSIF-V2) since it performs a good pH stability in fasted and fed state fluids. However it has been reported that maleate can interact with proteins. The -SH bond of proteins can irreversibly add across the double bond of maleic acid. It must also be noted that the buffers commonly used in dissolution media have low absorbance in the UV and visible region of the spectrum except for maleic acid (J. W. Mauger 2017).

Interestingly it has been further demonstrated that the surface tension has a significant impact on the quality of the medium (Fuchs & Dressman 2014). It is a pivotal parameter regarding the wetting of drug substances and their rate of dissolution. The choice of the bile salt along with the choice of phospholipid has shown a strong influence on this parameter (Fuchs et al. 2015). Furthermore the influence of sodium oleate and cholesterol have been demonstrated (Fuchs et al. 2015). Taking into account these data, the FaSSIF and FaSSIF-V2 media composition were reviewed in order to propose a third version (FaSSIF-V3) (Fuchs et al. 2015) (Table 5).

The phosphate buffer and maleic buffer were compared to investigate their impact on the surface tension and no significant effect was concluded. The target range values in human for the surface tension is 28 – 46 mN/m (Fuchs & Dressman 2014). Various prototypes of

FaSSIF-V3 were investigated regarding sodium oleate, lecithin, lysolecithin and different bile salts.

Table 5 Composition of the different fasted simulated intestinal media

	FaSSIF (Dressman, Amidon, et al., 1998.)	**FaSSIF** (Galia et al., 1998.)	(Vertzoni et al. 2004)	**FaSSIF-V2** (Jantratid et al. 2008)	**Copenhagen** (Kleberg, J. Jacobsen, et al. 2010)	**FaSSIF-V3** (Fuchs et al. 2015)
pH	6.8	6.5	6.5	6.5	6.5	6.7
Buffer type	phosphate	phosphate	maleate	maleate	trizma maleate	maleate
Osmolarity	280 – 310 mOsml	270±10 mOsmol	270±10 mOsmol/kg	180±10 mOsmol/kg	270 mOsm	220±10 mOsmol/kg
Bile Salt (NaTC)	5 mM	3 mM	3 mM	3 mM	2.5 mM	1.4
glycocholate	-	-	-	-	-	1.4
PL (Lecithin)	1.5 mM	0.75 mM	0.75 mM	0.2 mM	0.625 mM	0.035 mM
Lysolecithin	-	-	-	-	-	0.315 mM
BS/PL	3.3	4	4	15	4	
Pancreatin (lipase)	-	-	-	100 U/ml	-	-
Cholesterol	-	-	-	-	-	0.2 mM
Sodium Oleate	-	-	-	-	-	0.315

FaSSIF = Fasted state simulated intestinal media, NaTC = sodium taurocholate, PL = phospholipid

All these media yielded a surface tension in the targeted range although the medium which contained glycocholate (GC), taurocholate (TC), lysolecithin and sodium oleate showed a surface tension of about 34 mN/m (mean surface tension observed in the upper intestine). Therefore, this latter FaSSIF-V3 prototype was used to investigate the contribution of cholesterol and the measured value was slightly higher (35mN/m). Critical micelle concentration (CMC) was also tested. The ability of the medium to manifest a CMC proves its capacity to form micelles and thus to facilitate the solubilisation of hydrophobic drugs. According to this experiment a specific CMC could not be calculated for FaSSIF and FaSSIF-V2 but a total concentration of taurocholate (TC) and lecithin between 2 and 3 mM was required to start a micelle formation. On the other hand, the CMC of FaSSIF-V3 was measured to be 0.426 mM (Fuchs et al. 2015).

1.9.2.2. Fed state

The essential distinction between the fasted state medium and the fed state is the increase in levels of bile salts and phospholipids. The inclusion of free fatty acids and monoglycerides has been further employed (Grove et al. 2005; Sunesen, Pedersen, et al. 2005). In the intestine the pH is usually lower as compared to the fasted state. Fed state simulated intestinal fluid (FeSSIF) was initially developed in 1998 and reviewed and updated ten years later (FeSSIF-V2) (Dressman, Amidon, et al., 1998.; Galia et al., 1998.; Jantratid et al., 2008) (Table 6). Buffer capacity and osmolarity values were lower as well as the concentration of BS and PL which augmented the BS/PL ratio from 4 to 5. Additionally, in order to mimic the lipolysis of triglycerides by gastric and pancreatic enzymes, oleic acid (OA) and mono-olein (MO) were included (Jantratid et al. 2008). On the contrary in the Copenhagen medium the fasted and fed state pH remains at 6.5, the BS/PL ratio is 4 and the buffer component is maleate (Kleberg, F. Jacobsen, et al. 2010; Grove et al. 2005) (Table 6). Oleic acid and mono-olein were added to the fed medium to study their effect on surface tension but no changes were observed whereas the combination of all surfactant (*i.e.* BS, PL, OA, MO) had an effect. Despite the numbers of studies attempting to improve simulated media to better reproduce dissolution in human gastric and intestinal fluid, few of them could demonstrate a satisfying correlation between simulated and actual human intestinal fluid (Kleberg, F. Jacobsen, et al. 2010).

Table 6 Composition of the fed simulated intestinal media

	FeSSIF (Dressman, Amidon, et al., 1998.)	**FeSSIF** (Galia et al., 1998.)	**FeSSIF** (Vertzoni et al. 2004)	**FeSSIF-V2** (Jantratid et al. 2008)	**Copenhagen** (Kleberg, J. Jacobsen, et al. 2010)
pH	5	5	5	5.8	6.5
Buffer	acetate	acetate	citrate	maleate	maleate
Osmolarity	485 – 535 mOsm	635±10 mOsmol	635±10 mOsmol/kg	390±10 mOsmol/kg	-
Bile Salt (NaTC)	15 mM	15 mM	15 mM	10 mM	5 – 20 mM
PL (Lecithin)	4 mM	3.75 mM	3.75 mM	2 mM	1.25 – 5 mM
BS/PL	3.75	4	4	5	4
Sodium Oleate	-	-	-	0.8 mM	0 – 45 mM
Mono-Oleate	-	-	-	5 mM	0 – 10 mM

FeSSIF = Fed state simulated intestinal media, NaTC = sodium taurocholate, PL = phospholipid, BS = Bile salt

1.10. Statistical experimental design

Experimental study as opposed to observational study is a study in which the researcher voluntarily imposes a treatment on subjects to observe the response variable. In this type of study, the variables of interest are referred to as “factors” and are controlled so that data of their effect on a response variable can be obtained. Experimental study methods are extensively used in various research fields and especially in medicine and biology. For example, in our research study an experiment was designed to determine the effect of the different intestinal components on the solubility of drugs. The factors are the intestinal components and solubility is the response variable.

Statistical experimental design is the branch of statistics that allows the design and the statistical analysis of the experimental study. The key principles of the statistics in experimental design are replication, randomisation and blocking. Replication allows the estimation of experimental errors (sample standard deviation) and more explicit results (sample mean value). Randomisation means that each run is independent because it is not affected by the previous run and does not predict the subsequent run. Blocking aims at separating a known bias effect by grouping runs that are similar to one another.

The list of experimental design techniques is very long (*e.g.* Box-Behnken, central composite, Taguchi…) (Cavazzuti 2013) therefore in this introduction only the full factorial design and fractional factorial design are presented since these will be used in this study. Factorial experiments are a useful means of systematically studying interaction between factors and highlighting the most significant ones.

1.10.1. Full factorial design

In the full factorial design all combinations of the factor levels are considered. For instance, assuming three factors with *a* level for factor 1, *b* level for factor 2 and *c* level for factor 3, the study will involve a total of *abc* combinations. The most common factorial design is the two-level full factorial design where each factor has only two levels defined as “+1” for the high level (“H”) and “-1” for the low level (“L”) (Figure 11). Assuming *k* factors with two levels, the total number of runs is $N = 2^K$. One can also add central points of the design space. A central point is a run in which all the factors have the average value of their high and low levels and it is defined as “0”. The main advantage of the full factorial design is that main effects and interaction effects of the factors are not confounded. A confounded effect is when a change in the response variable cannot be distinguished between two variables. On the other hand, the disadvantage is that the number of runs will grow exponentially with the number of factors and levels (Cavazzuti 2013).

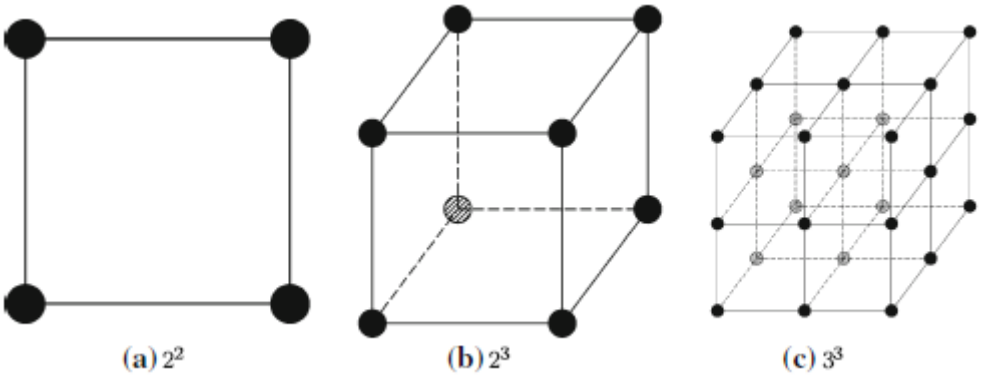

Figure 11 - Example of factorial design spaces – (a) two levels full factorial design with 2 factors (total measurements N= 2x2 =4) with a design space represented by a square – (b) two level full factorial design with 3 factors (total measurements N= 2x2x2 =8) with a design space represented by a cube – (c) three levels full factorial design with 2 factors (total measurements N= 3x3x3 =27) with a design space represented by eight cubes juxtaposed. NB: Fractional factorial design are difficult to represent with a 3D structure since they somehow represent a portion of the design space.

1.10.2. Fractional factorial design

Fractional factorial is a good alternative when the size of the full factorial becomes laborious and expensive to conduct. The principle of the fractional factorial design is to run only a subset or a "fraction" of the full factorial design (Cavazzuti 2013). The fraction can be one-half, one-quarter and so on, of the full one. Assuming a two-level fractional factorial design with *k* factors the one-half design is defined by 2^{K-1} runs, the one-quarter by 2^{K-2}, the one-eight by 2^{K-3} and so on (Figure 11 and Table 7). The advantage of the fractional factorial design is the reduced number of experiments however the resolution of the experiment is affected. The resolution expresses the extent of confounded effects and the most common designs are determined as follows with the higher the resolution number, the better the results (Table 7) (Cavazzuti 2013).

- Resolution of III : The main effects are confounded with two-factor interactions
- Resolution of IV: The main effects are not confounded with two-factor interactions, but two-factor interactions are confounded with each other
- Resolution of V : The main effects and two-factor interactions are not confounded but the two-factor interactions are confounded with three-factor interactions

Table 7 Example of a two-level factorial design with 8 factors (K = 8)

Design	**2^(*K* – p)**	**Runs**	**Resolution**
Full factorial	2^(8)	256	
½ fractional	2^(8 – 1)	128	VIII
¼ fractional	2^(8 – 2)	64	V
1/8 fractional	2^(8 – 3)	32	IV
1/16 fractional	2^(8 – 4)	16	IV

When performing the analysis of an experiment two types of effects are considered, main effects and interaction effects. The main effect is the effect of the variables of interest (factors) on the response variable. An interaction effect is the combined effect of two or more factors on the response variable. Assuming a two-level factorial experiment with two factors *A* and *B* thus four runs are required in total for the study. The main effect of *A* on the response variable is calculated by the difference between the average response when *A* is high and the average response when *A* is low. The difference of the response due to the change of level is called the main effect of the factor.

A (main effect) = (average response at A_{high}) – (average response at A_{low}) Equation 18

The interaction between the factors *A* and *B* is calculated according to the following equation:

AB (interaction) = average response at (A_{high} – B_{high} and A_{low} - B_{low}) –
average response at (A_{low} – B_{high} and A_{high} - B_{low}) Equation 19

The statistical analysis of the data from an experimental study generally uses an analysis of variance (ANOVA). Assuming the experiment where the effect of *A* and *B* factors was investigated on a response variable the ANOVA model is described as follow:

$Y_{ijk} = \mu + T_i + \delta_j + (T\delta)_{ij} + \epsilon_{ijk}$ Equation 20

where μ is the overall mean effect, T_i is the effect of the *i* th level of factor *A*, δ_j is the effect of the *j* th level of factor *B*, $(T\delta)_{ij}$ is the interaction effect between *A* and *B* and ϵ_{ijk} is the random term error. Hypothesis tests are then checked for each factor and interactions to determine whether they are significant or not. A multiple regression model is applied to determine the relationship between the factors and the response variable. In the case of factorial design, a linear relationship is always assumed between the factors and the response variable. The regression model is estimated by calculating the parameters of the model using the least square means.

1.11. Principles of pharmacokinetics

Pharmacokinetics is the study of the concentration of an administered drug inside an organism as a function of time[6]. A fundamental hypothesis is that the pharmacological effect of a drug is linked to its concentration in the systemic circulation. The pharmacokinetic studies include the different processes of absorption, distribution, metabolism and excretion of the drug known as ADME. The primary aspect of pharmacokinetics is the capacity of elimination of the xenobiotic (*i.e.* foreign substance) from the organism which is characterised by the clearance. Clearance is defined by the volume of fluid cleared of the drug per unit of time and is calculated as follows:

Clearance = dose / AUC Equation 21

Where AUC is the area under the plasma concentration curve (AUC) reflecting the total drug exposure over time. As shown in the equation above, clearance is inversely proportional to the AUC. Clearance can also be described as a function of both distribution and elimination and calculated as follows:

Clearance = $V K_e$ Equation 22

where *V* is the volume of distribution that is the volume of organism fluid in which the dose is distributed and K_e is the elimination rate constant. Many drugs are cleared from the organism

[6] Holland-Frei Cancer Medicine. 6th edition. Principles of pharmacokinetics. Mark J. Ratain, MD and William K. Plunkett, Jr, PhD

with a first-order kinetic process. In that case there is a linear relationship between the concentration of the drug and its elimination rate therefore the elimination rate is constant. The elimination rate constant K_e is also inversely proportional to the half-life. Half-life is the time needed to reduce by 50% the concentration of the drug in the plasma. The smaller the half-life, the higher the decline in plasma concentration and thus the elimination.

For drugs administered *per os* (*i.e.* via the oral route) gastro-intestinal absorption is an essential parameter as it will determine the fraction of the administered dose that reaches the general circulation (F) for the expected therapeutic effect. In this case equation 21 can be written as follows:

$$\text{Clearance} = F * \text{dose} / \text{AUC} \qquad \text{Equation 23}$$

1.11.1. Linear pharmacokinetics

Linear pharmacokinetics is defined by a proportional relationship between the dose administered and the clearance (elimination). Clearance is therefore not dependent on the dosing schedule and half-life is independent of the concentration. It is featured in the following equation:

$$dC/dt = -K_eC \qquad \text{Equation 24}$$

This equation shows that the rate of change in drug concentration is directly proportional to the concentration present. The elimination half-life will stay constant even with high concentrations. This principle implies that drug schedule will not affect the drug exposure (AUC) and that drug exposure is proportional to the dose.

1.11.2. Non linear pharmacokinetics

Nonlinear pharmacokinetics occurs when one of the pharmacokinetic processes is saturated. The two affected processes can be absorption and/or elimination. Saturated elimination means that above a given drug concentration the elimination reaches a maximal value. Any further increase in plasma concentration will not increase the elimination rate therefore the drug clearance will not increase with the plasma concentration. Saturated absorption means that above a given drug concentration the absorption reaches a maximal capacity. The absorption rate and bioavailability will decrease with increasing doses. In addition, protein binding or drug reabsorption in kidney tend to saturate above a certain drug concentration. This leads to a disproportional relationship between the drug concentration and its rate of

elimination. The clinical implication for drugs with nonlinear pharmacokinetic is the important influence of administration schedule on the area under the curve (AUC) compared to drug with linear pharmacokinetics. In the case of non-linearity changing the dose can be unpredictable since an increase in dosage may lead to a disproportionate increase in plasma concentration.

1.11.3. Inter and intra-individual pharmacokinetics variability

1.11.3.1. Inter-individual variability

When investigating the pharmacokinetics of drugs, it is important to consider the inter-individual variability which is often presented as the coefficient of variation (CV). Analysing the potential cause of differences between individuals can be very useful in understanding the variability. In the case of cancer patients, various patient conditions can lead to anomalies of absorption or distribution (Table 8). Furthermore, variations in pharmacogenetics especially in metabolism profiles have been reported as crucial factors in the variability of pharmacokinetics (Wasserman et al. 1997).

Table 8 Potential causes of inter-individual variability in the oncologic population

Anomalies of absorption	**Anomalies of distribution**
Nausea/vomiting	Weight loss
Prior surgery, radiotherapy or chemotherapy	Obesity
Antiemetic affecting gut motility	Decreased body fat (lipophilic drugs)
Patient compliance	
Concomitant medications	

(Holland-Frei Cancer Medicine. 6th edition. Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Hamilton (ON): BC Decker; 2003)

Variability in distribution is influenced by variations in body size or total body fat. The latter case will have a direct impact on the most lipophilic drugs. For example, due to its high lipophilicity methotrexate can accumulate in ascites or pleural tissue and be slowly released. This results in a delayed clearance of methotrexate (Chabner et al. 1978).

1.11.3.2. Intra-individual variability

Intra-individual variability is the variability observed within one single subject. Circadian rhythm is a potential source of variability within individual subjects. It has been demonstrated that a two-fold difference of the plasma concentration of 5-FU is observed between the maximum and minimum values when 5-FU is infused during 5 days at a constant rate (Petit et al. 1988).

1.12. Principles of physiologically based pharmacokinetics

Pharmacokinetic modelling usually applies an empirical method to describe the fate of a drug in the body represented by separate compartments (Gerlowski & Jain 1983). Typically for an oral formulation the drug is transferred from the site of administration (absorption) to a central compartment where it can be distributed to peripheral compartments (distribution) and eliminated (metabolism and excretion). Absorption and elimination are defined by the rate constants Ka and Ke, respectively (Figure 12). A compartment is an entity defined by a specific volume with a uniform distribution (Gerlowski & Jain 1983).

Compartmental modelling is descriptive and interprets the pharmacokinetics of a drug based on observations and allows predictions and adaptation of dose regimen (Gerlowski & Jain 1983). It is called a “top-down” approach as opposed to the “bottom-up” methods represented by PBPK modelling. The bottom-up approach describes the physiological processes in a mechanistic way to mimic the biology. The models consist of compartments representing the different organs linked together by the blood circulation (Figure 12). Full PBPK models incorporate all the main organs of the organism while semi-PBPK models use a pooling compartment in which different organs are represented. Each compartment is defined by a tissue volume and a blood flow rate and each tissue is described as either perfusion or permeability rate limited. In a perfusion rate-limited tissue the blood flow to the tissue is the limiting process. In a permeability rate-limited tissue the permeability accros the cell membrane becomes the limiting process (Upton et al. 2016). The drug is then eliminated via the blood circulation. The limitations of compartmental models have led to an extensive development of PBPK models alongside compartmental models.

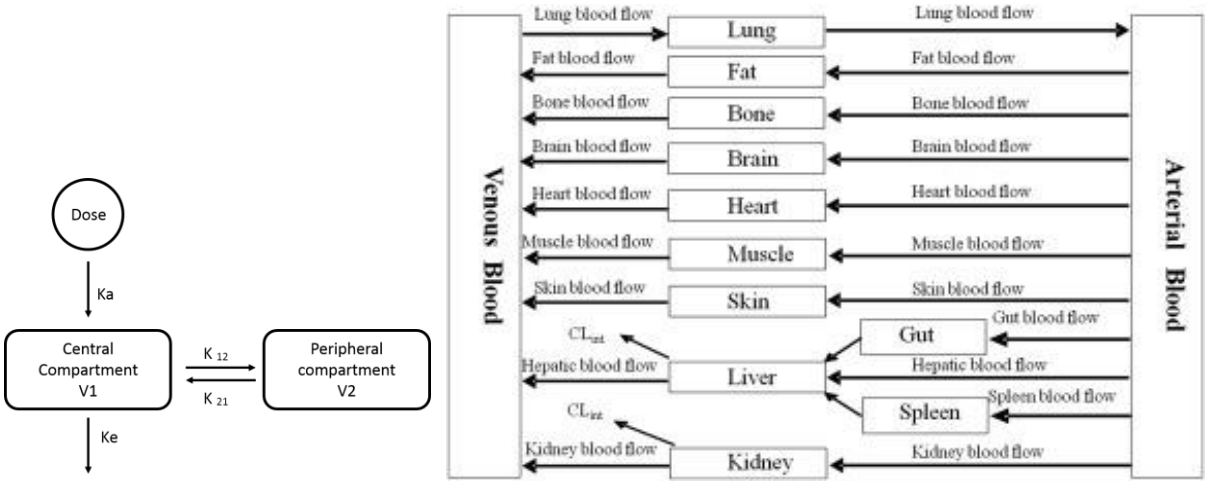

Figure 12 - Schematic of an empirical “classical” model (left) and a mechanistic PBPK model (right) (Zhuang & Lu 2016)

The parameters composing a PBPK model are either drug-dependent or drug-independent. The drug-independent parameters constitute the frame which is the anatomical physiology relative to the species of interest (animal or human) and therefore can be applied to any drug (Upton et al. 2016). The drug-dependent parameters are the specific physicochemical and ADME properties. As a consequence, the performance of the model predictions will depend on both the organism physiology and the input biochemical properties of the drug.

Several commercial PBPK platforms have been developed with three-leading modelling software which are GastroPlus™ (Simulation Plus, Inc., Lancaster, CA), Simcyp® simulator (Certara, USA), and PK-Sim® (Open Systems Pharmacology Suite). The main applications of PKPB models for drugs orally administered are the prediction of food effect (Parrott et al. 2009), the study of different formulations and the potential causes of poor bioavailability. In addition, they allow the study of drug-drug interactions (DDI) (Yeo et al. 2013), hepatic and renal impairment (Edginton & Willmann 2008) and scaling to specific populations such as the paediatric population (Khalil & Läer 2014).

1.12.1. Absorption models from “CAT to ADAM”

The absorption model of those three modelling platform is based on the original Compartmental Absorption and Transit model (CAT) first described by Yu et al. (Yu et al. 1996). In this model the different sections of small intestine are represented by seven compartments. The duodenum, the upper and lower jejunum, and then four compartments for the ileum. The mathematical model is very simple since transit between compartments is defined by a single rate constant. Further improvement of the CAT model led to the Advanced Compartment Absorption and Transit model (ACAT). The development of this model includes the addition of the stomach and colon compartment to incorporate processes like delayed

gastric emptying and colonic absorption. However, the main enhancement is the implementation of three drug states in each segment: the unreleased drug in formulation, the undissolved and the dissolved drug. This allows the study of the effect of the formulation. Following the ACAT model the most prominent ameliorated version is the Advance Dissolution Absorption Metabolism model (ADAM) implemented in the Simcyp simulator (Jamei et al. 2009). The structure remains identical with seven compartments plus the stomach and the colon but a supersaturation state of the dissolved drug is permitted. This phenomenon is particularly important for a weak base with pKa < 7 when the pH-dependent solubility drops significantly between the fasted stomach and the duodenum.

1.12.2. Absorption model in PK-Sim®

The intestinal absorption model implemented in PK-Sim is made of 12 compartments (Figure 13). They describe the lumen of the GI tract from the stomach to the rectum (stomach, duodenum, upper and lower jejunum, upper and lower ileum, caecum, ascending-transverse-descending-sigmoid colon and rectum) with varying properties such as dimensions, surface area and pH. In each compartment the lumen is divided in two sections, the fluid volume of the compartment (Liquid) and the drug in solution (DIS). The drug in solution (DIS) of each compartment is linked to the DIS of the following luminal compartment and also to the compartment of the intestinal wall parallel to it which is the mucosa. The mucosa of each compartment is subdivided in four sections depicting the intracellular (cell), the interstitial (INT), the red blood cells and the plasma. The mucosa was implemented independently to allow a more realistic description of the absorption processes and a more efficient modelling of the gut wall metabolism (Thelen et al. 2011). The first model was developed to simulate the absorption process of oral solutions but to account for the administration of solid dosage forms (*e.g.* tablet) the model was further revised (Thelen et al. 2012). An independent species was added (Solid) to model the GI transit and disintegration. After being emptied from the stomach, the solid form can be transported at various rates within the intestine. The solid can be released in the luminal fluid according to the seven different dissolution functions available. The drug in solution species is then available to be transported along the different compartments. The absorption of solid forms is governed by its dissolution and solubility in the GI tract. In PK-Sim® the pH-dependent solubility for ionisable drugs is described using the Henderson-Hasselbalch equation. In addition, the input of a single value in biorelevant media is allowed (*e.g.* FaSSIF or FeSSIF) however, the software does not account for the calculation of the bile dependent solubility.

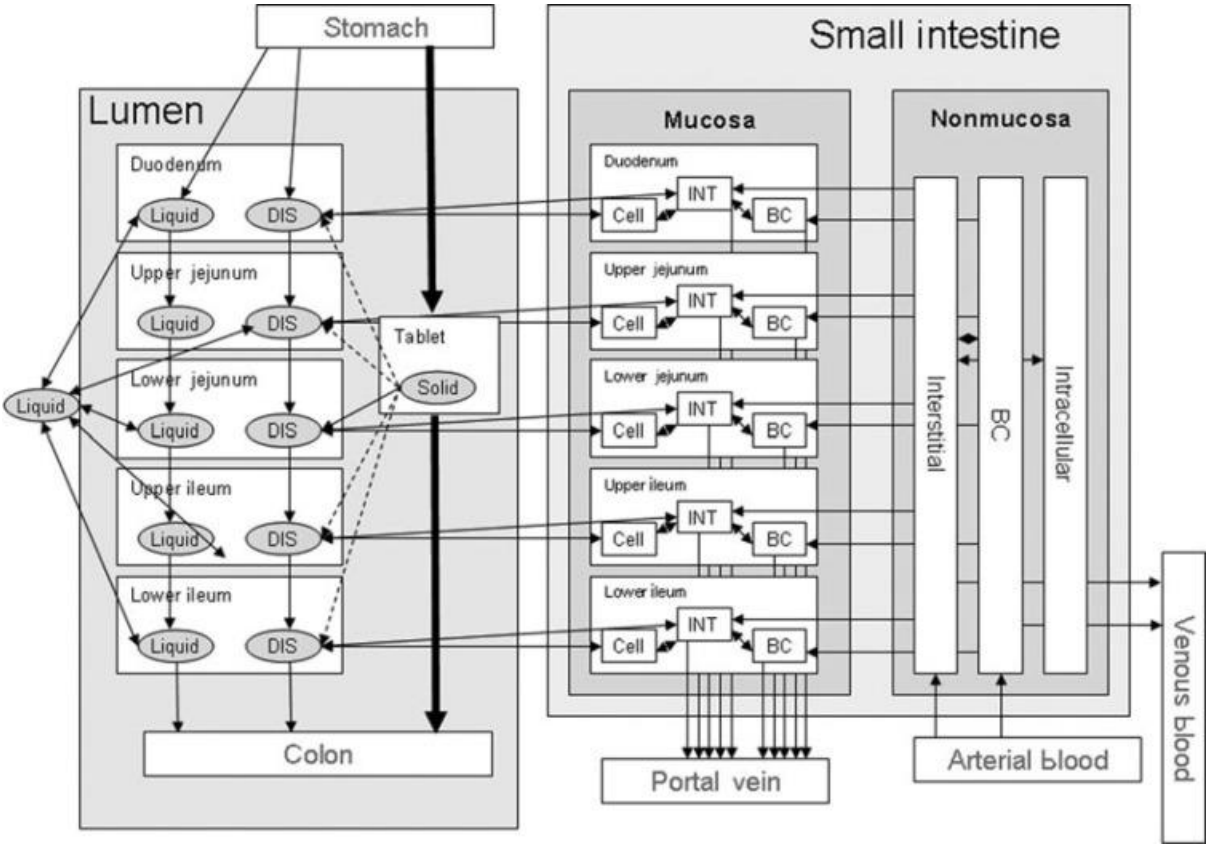

Figure 13 - Structure of the transit and absorption model implemented in PK-Sim. The large intestine has the same structure but is not shown for better visualisation (Thelen et al. 2012)

1.13. Aim and objectives of this research project

The aim of this project was to gain a better understanding and characterisation of the solubility and absorption in the human intestinal tract of poorly soluble drugs administered via the oral route. It is believed that up to 70% of the therapeutic drugs in the development process display a poor water-soluble profile and as a consequence are very unlikely to reach the market. Since most drugs are intended to be taken orally as capsules or tablets, water solubility is an essential requirement. In order to address this concern the Innovative Medicines Initiative (IMI) initiated a collaborative project involving public and private partners such as EFPIA[7] pharmaceutical companies, universities and research organisations within Europe. The aim of the project was to develop new Oral Biopharmaceutical Tools (OrBiTo) to enhance the understanding of how oral drugs are taken up from the gastrointestinal tract and accelerate the development of new medicines.

[7] European Federation of Pharmaceutical Industries and Associations

The research project presented herein contributed to this effort since the first objective was to develop an *in vitro* tool to investigate the human intestinal solubility.

Following the general introduction the second chapter is focused on the investigation of new medium simulating the human intestinal fluid and its influence on the solubility of poorly soluble drugs. A biorelevant medium was created using the most important intestinal components reported in the literature to simulate *in vitro* the pre- and post-prandial states. A design of experiment statistical method was employed to study the equilibrium solubility of drugs with various ionisation properties and to identify the influence of the media components.

The objective of the third chapter is to demonstrate the usefulness of the DCS classification to assist the formulation development. Different case study are presented and the potential utility of applying the DCS during the development process is discussed.

The fourth chapter is focused on the computational prediction of biorelevant solubilities of poorly soluble drugs and the comparison of the performance of two modelling software. In this chapter the *in vivo* behaviour of the drugs is also studied using a physiologically based pharmacokinetic modelling method and the direct impact of the input solubility is evaluated.

In the last chapter (chapter five) the objective was to apply the *in vitro* and *in silico* knowledge gathered in the previous experiments to build a PBPK model of a cancer drug. Data of the dose escalation study in the phase I clinical trial were available for this drug therefore the *in vitro* solubility test was applied to characterise its intestinal solubility and then the PBPK model was built to check whether the dose escalation study could be reproduced *in silico*.

Chapter 2

Stastistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs

2. Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs

2.1. Introduction

Dissolution and solubility are essential parameters in the absorption process of orally administered drugs and especially for poorly soluble drugs (BCS class II and IV). Over the last two decades there has been an increasing development of molecules with low aqueous solubility due to the application during development of high throughput screening systems (Lipinski et al. 1997). Therefore, it is necessary to develop new formulation techniques in order to address this issue (Savjani et al. 2012) along with *in vitro* methods to predict drug solubility in gastrointestinal fluids (Lennernäs et al. 2014). High throughput solubility screening is possible (Alsenz & Kansy 2007) since a low aqueous solubility does not automatically mean poor gastrointestinal solubiliy. The solubilising potential of the gastrointestinal environment can improve the bioavailability for some drugs over that predicted on the basis of simple aqueous solubility (Sunesen, Pedersen, et al. 2005). For example, it has been reported that mixtures of bile salts increase the solubility of steroid formulations (Mithani et al. 1996; Wiedmann et al. 2002) and the interaction of lecithin with bile salts yields an even greater positive solubility effect (Naylor et al. 1993). Solubilisation can be further influenced by the formation of mixed micelles with other lipid digestion products such as monoglycerides and the interaction of monoglycerides with bile salt was demonstrated to increase the solubility of alpha-tocopherol in comparison to bile salt alone (Nielsen et al. 2001). To address the problem of poor aqueous solubility and bioavailability for oral drug formulations, it is therefore essential to use solubility and dissolution test conditions which closely reproduce key parameters of human gastrointestinal physiology (Dressman et al. 2007). Over the past two decades simulated gastrointestinal media for the human fasted and fed states have been developed to assist *in vitro* drug development and formulation studies (Markopoulos et al. 2015; Stappaerts et al. 2014). These media were based around available literature data on the detailed composition and physicochemical parameters of human GI fluid however, the gastrointestinal tract and the interactions of all its constituents is very complex. To assess these interactions and improve the determination of the pivotal factors influencing the intestinal solubility of BCS class II drugs, a statistical design of experiment (DoE) approach was applied to investigate the influence of simulated gastrointestinal media composition in the fasted (Khadra et al. 2015) and fed state (Zhou et al. 2017b) on the equilibrium solubility of BCS II compounds. This illustrated the utility of this approach, provided solubility values that are in agreement with literature values and highlighted the differences in solubility between the fasted and fed state (Augustijns et al. 2014; Bevernage et al. 2010; Clarysse et al. 2011). In addition, the approach simulated the inherent solubility variability and determined the key

parameters controlling a drug's solubility. For acidic drugs pH was the most significant factor. For basic and neutral drugs the combination of pH and concentration of sodium oleate, bile salt and lecithin was significant. Various interactions between media components and unusual drug specific solubility behaviour were also identified. For neutral drugs solubilisation in fed simulated media was a more complicated interplay since seven (pH, oleate, bile salt, lecithin, monoglyceride, buffer and pancreatin) out of the eight single factors were significant along with more than half of the factor interactions. In this study the design of experiment approach has been applied to explore the equilibrium solubility of BCS class II drugs in simulated media spanning the full range of both fasted and fed intestinal states in a single experiment. The purpose is to examine the feasibility of merging the individual fasted and fed studies into one reduced experiment in order to obtain comparable results from a smaller experimental load. In this full range DoE the simulated intestinal fluid consists of seven factors or parameters (sodium oleate, bile salt, pH, lecithin, buffer, salt and monoglyceride) with phosphate buffer used instead of maleic acid. A fractional factorial design with two levels (upper and lower limit) was applied requiring a total of thirty two measurements and conducted in duplicate. This gives a total of 64 measurements for the statistical analysis. The lower limit values are derived from the lower limits of the literature fasted study (Khadra et al. 2015) and the upper limits are from the upper limits of the fed study (Zhou et al. 2017b) (Table 9). This smaller scaled DoE was selected in order to assess the utility of this systematic approach with a limited number of measurements. The equilibrium solubility of nine BCS class II drugs was investigated, two acids (indomethacin and phenytoin), four bases (aprepitant[8], tadalafil, zafirlukast and carvedilol) and three neutral drugs (felodipine, fenofibrate, probucol) (Table 10) and compared to the previous fasted and fed DoE studies.

[8] Aprepitant has been classified as a basic drug in order to assist comparison with the two previous design of experiment studies, it is recognised that with its reported pKa values it will be mostly non-ionised over the studied pH range.

Table 9 Composition and concentration levels employed in the full range design of experiment

parameter	substance	Lower limit fasted (mM)	Upper limit fed (mM)
Bile salt	Sodium taurocholate	1.5	24
Lecithin	Phosphatidylcholine	0.2	4.8
Fatty acid	Sodium oleate	0.5	52
pH	Sodium hydroxide/hydrochloric acid	5	7
Salt	Sodium chloride	68	203
Buffer	Phosphate [a]	15	45
Monoglyceride	Glycerol mono-oleate	0.5	6.5

[a] Monophosphate buffer (KH_2PO_4)

2.2. Materials and methods

2.2.1. Materials

Sodium taurocholate, ammonium formate, sodium chloride (NaCl), chloroform, formic acid, monosodium phosphate (NaH2PO4), fenofibrate, indomethacin and phenytoin were purchased from Sigma Aldrich Poole, Dorset UK. Lecithin S PC (phosphatidylcholine from Soybean “98%”) was purchased from Lipoid.com. Glycerol mono-oleate was obtained from CRODA Healthcare. The active pharmaceutical ingredients felodipine, probucol, aprepitant, tadalafil, carvedilol and zafirlukast were provided through OrBiTo by Dr. R. Holm Head of Preformulation, Lundbeck, Denmark. Sodium oleate was obtained from BDH Chemical Ltd. Poole England. The analytical solvents methanol and acetonitrile were of HPLC grade (VWR, UK). The water used was ultra pure Milli-Q water.

Table 10 Physicochemical properties of the studied compounds

compound	pKa	MW	LogP	S_0	Tm	PSA
Indomethacin	4.5 (a)	357.7	4.2	0.0065	151	68.5
Phenytoin	8.3 (a)	252.2	2.4	0.010	286	58.2
Carvedilol	7.8 (b)	406.4	4.2	0.010	114.5	75.7
Tadalafil	10 (b)	389.4	1.7	0.018	302	74.9
Zafirlukast	4.3 (b)	575.6	5.4	0.0024	139	124
Aprepitant	2.8 (b) / 9.7 (a)	534.4	4.5	0.0019	252	75.2
Felodipine	Not ionised	384.2	3.8	0.011	145	64.6
Fenofibrate	Not ionised	360.8	5.2	0.0047	80.5	52.6
Probucol	Not ionised	516.8	10	2.7E-5	125	91.1

All data taken from Pubchem otherwise mentioned

(MW) molecular weight in g/mol

(logP) lipophilicity octanol/water

(Tm) melting temperature in degrees celcius

(PSA) polar surface area in Angstrom (A^2)

(a) for acidic pKa, (b) for basic pKa.

Intrinsic solubility (So) in molar concentration calculated with the GSE equation as follows: $\log So = -0.01\,(Tm-25) - \log K_{ow} + 0.5$ (Ran & Yalkowsky 2001)

2.2.2. Design of experiment and data analysis

A quarter of the full factorial design of experiment with 7 factors (either a component concentration or a system parameter such as pH) and 2 levels (upper and lower limits) was constructed and analysed using Minitab® version 17.2.1. By combining the upper and lower limits of the 7 factors based on Table 9 Minitab generated 32 different media with one replicate for each medium. It resulted in a total of 64 solubility measurements to perform (no centre point). When designing and analysing the DoE assumptions were made. 1. Only main effects and 2-way interactions are considered in the analysis, 3-way interactions or more were not considered. 2. The single factors and factor interactions are confounded with 3 to 6-way interactions which were not included. There are three confounded 2-way factor interactions, sodium oleate and salt with buffer and monoglyceride, sodium oleate and buffer with salt and

monoglyceride, sodium oleate and monoglyceride with salt and buffer. For these interactions if the result is significant then any conclusions must be drawn with caution as it might be the result of the four factors together or only one of the 2-way interactions. 3. The main effect can be positive (+) or negative (−), but when it is involved in an interaction, the conclusion will be considered with the interactions (±). The Kolmogorov-Smirnov normality test was applied in Minitab® to assess the distribution of each data set. The level of significance was set to 0.05 therefore if the p-value was less than 0.05 the distribution was non-normal. The Mann-Whitney non-parametric test was applied to evaluate differences between two data sets. The level of significance was set to 0.05 to calculate whether the medians of two data sets differ. If the p-value was greater than 0.05 the two medians were not significantly different (displayed as NS on the graph). If the p-value was less than 0.05 the two medians were significantly different (displayed on the graph as * if p-value ≤ 0.05; ** if p-value ≤ 0.01; *** if p-value ≤ 0.001 and **** if p-value ≤ 0.0001).

2.2.3. Equilibrium solubility measurements

2.2.3.1. Preparation of lipid stock mixtures

Sodium taurocholate, monoglyceride and lecithin were weighed into a flask and 2 ml of chloroform was added to dissolve all the solid material. A stream of nitrogen gas was used to remove the chloroform ensuring a dry film was produced. Water was added to reform the dried film, stirred to obtain a homogenous mixture, transferred to a volumetric flask (5 ml) and made to volume with water.

2.2.3.2. Preparation of aqueous stock solutions

Salt and buffer stock solution: Sodium chloride (4.448 g) and monosodium phosphate (NaH_2PO_4) (2.395 g) were weighed into a 25 ml volumetric flask, dissolved and made up to the volume with water.

Sodium oleate: Sodium oleate (1.978 g) was weighed into a 25 ml volumetric flask, dissolved in water under gentle heat and made to final volume. Solution was then kept at 50 °C to aid solubilisation.

2.2.3.3. Preparation of measurement solutions

The concentration of each stock mixture has been designed to be 15 times greater than the upper limit concentration value required for the DoE, with the exception of sodium oleate where only a 5 times concentration was possible. The stock mixtures were combined to provide the 32 measurement solutions according to the DoE model.

2.2.3.4. Determination of equilibrium solubility

This protocol has been previously validated to ensure equilibrium solubility is achieved after 24 h with no methodological interference (Khadra et al. 2015; Zhou et al. 2017b). An visual excess of powdered drug (around 10 mg) was added to a centrifuge tube (15 ml Corning®). The excess amount of 10 was set empirically based on the solubility observed in the previous studies. The required volume of each stock solution (section above) and water was a mg dded to provide a final volume of 4 ml in every tube. pH was then adjusted to 5 or 7 using 0.1M HCL or 0.1M KOH. Tubes were shaken on an orbital shaker for 1 h at room temperature and then pH adjusted again as before. Tubes were then placed in a tube rotator at 40 rpm, and incubated at 37 °C for 24 hours. Following incubation the tubes were checked for the presence of solid drug, then centrifuged (13,000 rpm, 5 min) and the supernatant (500 µl) was sampled to determine the solubilised drug concentration by HPLC. A single measurement of each tube was performed which resulted in 64 solubility points per drug (34 media in replicate). HPLC assay conditions are presented in Table 11.

Table 11 HPLC assay conditions

Column	Drug	Mobile phase	Flow rate (ml/min)	Injection volume (µl)	Detection (nm)	Retention time (min)	R^2	RSD (%)	LLOQ (µM)
2	Indomethacin	Mobile phase A: Ammonium formate 10 mM pH 3.0 in H2O Mobile phase B: Ammonium formate 10 mM pH 3.0 in Acetonitrile/H2O (9:1 v/v)	1	10	254	0.84	0.991	2.3	0.31
2	Phenytoin		1	10	260	2.3	0.996	2.5	51
1	Felodipine		1	10 – 50	260	3.1	0.999	4.6	9.4
1	Fenofibrate		1	10	291	3.7	0.994	5.0	0.94
1	Probucol		1	10 – 50	254	4.5	0.999	2.4	2.5
1	Aprepitant		1	10	254	3.0	0.999	2.4	26
2	Tadalafil		1	10	291	2.1	0.998	4.8	1.9
2	Zafirlukast		1	10	260	2.9	0.999	3.8	0.27
2	Carvedilol		1	10	254	1.0	0.999	3.8	9.0

The HPLC method is a generic method developed in collaboration with Mark Fever and Melvin Eureby from Hichrom Ltd. It has been validated for thirteen pharmaceutical compounds (including the ones studied here) and is available as a draft for publication. (Title: “Generic RP-HPLC method for the simultaneous determination of multiple API’s”)

Apparatus: Agilent Technologies 1260 Series Liquid Chromatography system with Clarity Chromatography software

Method: Gradient method: Time 0, 70%A:30%B, 3 min 0%A:100%B, 4 min 0%A:100%B, 4.5 min 70%A:30%B total run time 8 min.

Column 1 Hichrom ACE 3 C18/DV148262/50×3.0mm id/ACE-111-0503/A149937 / Column 2 Hichrom ACE 3 C18/SIN-A46224/50×2.1 mm/ACE-111-0502/A46224

R^2 : Linear regression coefficient of calibration curve, N=5 or 6 points.

RSD: relative standard deviation for Intra-assay precision (repeatability). All values are below 5% in compliance with ICH guidelines “Validation of analytical procedures” (1996)

LLOQ: Lower Limit of Quantification determined with the Signal-to-Noise approach. A single to noise of ratio of 3 was considered acceptable for estimating the detection limit.

2.3. Results and discussion

2.3.1. Equilibrium solubility measurements

The results of the full range DoE equilibrium solubility measurements are presented in Figure 14 and a broad range of solubility values are observed with heterogeneous variability from one to three orders of magnitude depending on the drug. As a comparison literature solubility values were available for six drugs in fasted or fed state simulated intestinal fluid (SIF) and/or human intestinal fluid (HIF) (Augustijns et al. 2014) and are plotted in Figure 14. Those results are comparable in each case and lie within the DoE range of the solubility values reported in this study. It is evident that drug specific factors are affecting solubility with some compounds felodipine and tadalafil showing a large variability while phenytoin and aprepitant show more consistency.

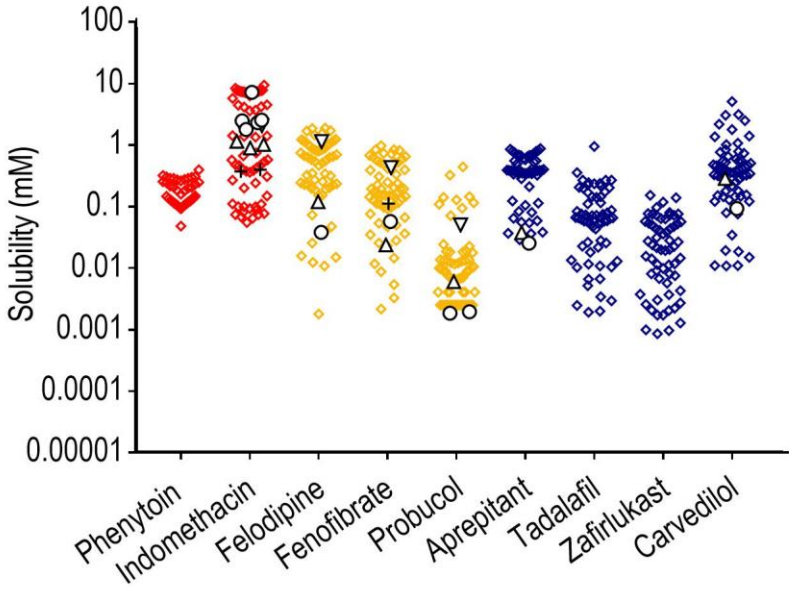

Figure 14 - Design of experiment equilibrium solubility measurements. Equilibrium solubility measurements for each drug based in DoE media compositions detailed in Table 9. Red coloured data points for acidic drugs, orange for neutral drugs and blue for basic drugs. Δ and + reported solubility values for individual drugs in fasted simulated intestinal fluid and fed simulated intestinal fluid media respectively (FaSSIF, FeSSIF), o and ∇ reported solubility values for individual drugs in fasted human intestinal fluid (FaHIF) and fed human intestinal fluid (FeHIF) respectively, all values from (Augustijns et al. 2014)

In Figures 15 – 17 the published fasted (Khadra et al. 2015) and fed (Zhou et al. 2017b) DoE measurements are plotted for each drug along with the full range data and displayed by group.

For acidic drugs (Figure 15) the concentration points are comparable with the previous fasted and fed studies with the solubility of phenytoin very consistent while indomethacin exhibits a larger variability. The respective pKa of the drugs 8.3 and 4.5 could explain this difference since phenytoin is unionised over the experimental pH range whilst indomethacin is predominantly ionised. In addition indomethacin is more lipophilic (log P=4.27) which will increase its interaction with the micellar phase. For the basic drugs (Figure 16) tadalafil and carvedilol the previous fasted and fed data are comparable to the full range experiment whilst zafirlukast and aprepitant do not show the same consistency. The full range DoE was not able to determine the lowest concentrations for both drugs. Zafirlukast has the biggest difference between the lowest fasted and highest fed values (4 orders of magnitude). In addition the distribution of the full range of data points is the most homogenous compared to the other distributions. This compound has the highest log P value (5.4) and a pKa of 4.3 which means that the non-ionised form is predominant over the pH range. Carvedilol and tadalafil are largely ionised between pH 5 and 7 according to their respective pKa values of 7.8 and 10 while the ampholyte aprepitant is considered as a neutral compound between pH 5 and 7. The lipohilicity of aprepitant (log P=4.5) and carvedilol (log P=4.2) could explain the slightly higher solubility observed. For neutrals (Figure 17) depending on the drug the full range experiment was able to determine very low concentrations. Neutral compounds are not ionisable therefore lipophilicity plays an important role in the solubilisation by surfactants and micelles. Felodipine and fenofibrate (log P=3.8 and 5.3 respectively) behave similarly since the full range covered the fasted and fed space and the lowest concentration corresponds to the lowest point of the fasted experiment. However, the solubility of probucol is lower which may indicate that its very high lipophilicity (log P=10) might limit solubilisation. Interestingly the measured equilibrium solubility values indicate that the full range DoE covered the solubility space of the previous fasted and fed DoE for the majority of the drugs. This outcome means the full range DoE is covering an appropriate solubility space and that a reduced experimental size DoE could be sufficient to explore the intestinal solubility variability in simulated media.

2.3.2. Statistical comparison

All the data sets resulted in a non-normal distribution, which based on the number of data points (fasted DoE=66 (Khadra et al. 2015), fed DoE=92 (Zhou et al. 2017b), full range=32) was not expected and may arise either through the non-normal sample pattern induced by the DoE structure and / or the fact that drug solubility is not normally distributed in the sample space. The latter explanation is supported by human intestinal fluid characterisation studies which indicate that bile salt and lecithin in the fasted state have skewed concentration

distributions (Riethorst et al. 2015). Human intestinal fluid solubility studies measured differences between mean and median solubility values (Psachoulias et al. 2011) indicate a non-normal solubility distribution.

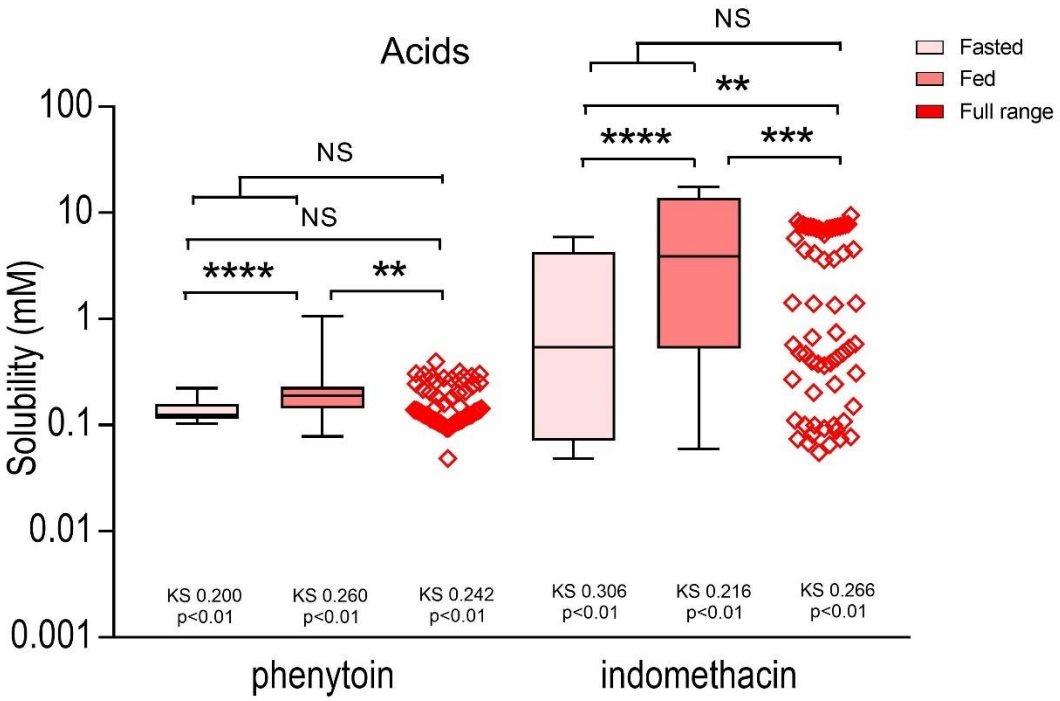

Figure 15 - Design of experiment equilibrium solubility measurements – Acids. Box-whisker plots for fasted and fed solubility data distribution respectively; from top to bottom the maximum value, 75th percentile, median, 25th percentile and minimum value. Scatter plot for individual data points of full range solubility distribution.

Further studies will be required to fully explore this interesting statistical property. A non-parametric Mann-Whitney test was therefore applied to compare distributions and the p-values are displayed in each figure. It is evident for all the drugs that the solubility values are lower in the fasted than the fed state which is in agreement with the literature data (Augustijns et al. 2014; Bevernage et al. 2010; Clarysse et al. 2011) and indicates that the published DoE (Khadra et al. 2015; Zhou et al. 2017b) studies have explored different solubility spaces. A comparison of the published fasted or fed solubility distributions with the current full range DoE indicates that there is a statistically significant difference for fourteen (approximately 80%) out of the possible eighteen

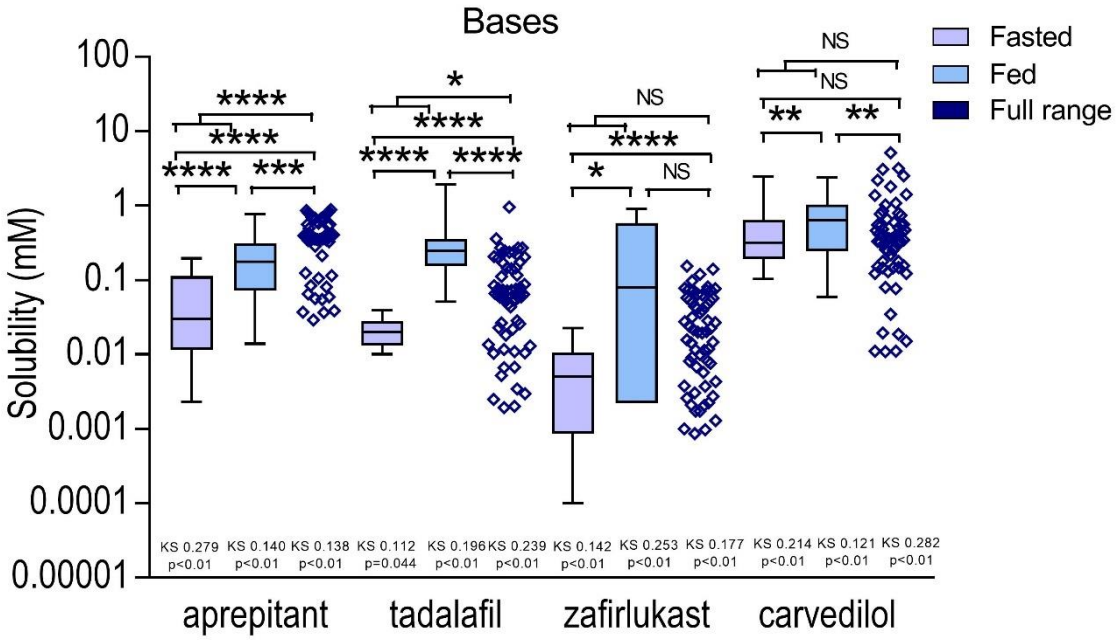

Figure 16 - Design of experiment equilibrium solubility measurements – Bases. Box-whisker plots for fasted and fed solubility data distribution respectively; from top to bottom the maximum value, 75th percentile, median, 25th percentile and minimum value. Scatter plot for individual data points of full range solubility distribution.

(two for each of the nine drugs tested) comparisons. In the fasted state phenytoin and carvedilol and in the fed state zafirlukast and felodipine are statistically equivalent to the combined DoE. The difference between fasted or fed compared to combined is to be expected based on the previous comparison between fasted and fed, which determined that these are separate solubility distributions. In the case of phenytoin and carvedilol in the fasted and felodipine in the fed DoE the similarity can be ascribed to the narrow solubility distribution, which fits inside the full range distribution, whilst for zafirlukast there is a broad overlapping between fed and full range. A comparison of the combined fasted and fed solubility data, which if additive should represent the full solubility range, with the full range DoE indicates that there is no significant difference for six (phenytoin, indomethacin, zafirlukast, carvedilol, fenofibrate, probucol) out of the nine drugs tested but aprepitant, tadalafil and felodipine are significantly different. The statistical equivalence between a combination of published fasted and fed data with the full range DoE data is to be expected if both experiments are sampling the same solubility space.

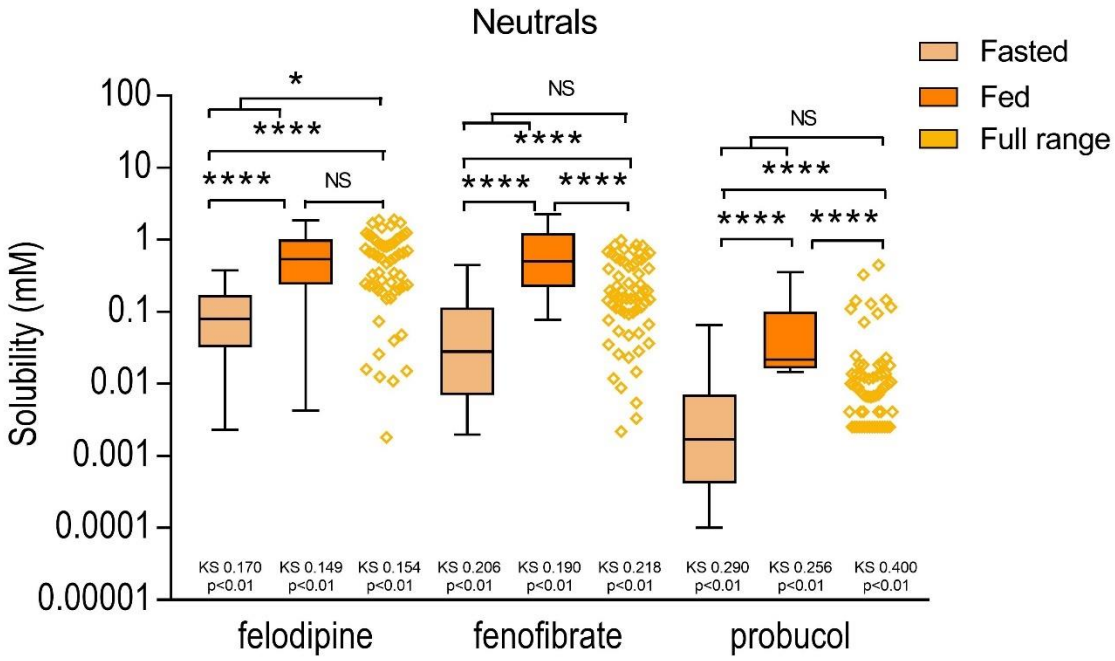

Figure 17 - Design of experiment equilibrium solubility measurements – Neutrals. Box-whisker plots for fasted and fed solubility data distribution respectively; from top to bottom the maximum value, 75th percentile, median, 25th percentile and minimum value. Scatter plot for individual data points of full range solubility distribution.

The statistically significant difference determined for aprepitant, tadalafil and felodipine appears to be related to the trend towards higher “fed” like solubility values in the full range DoE, when compared to the solubility for the combined fasted and fed data. However, for aprepitant and tadalafil there is a statistically significant difference between all data sets (Figure 16) indicating that these drugs are exhibiting complex behaviour. This discrepancy in one third of the tested drugs might be due to the aforementioned issue that the application of a DoE approach samples the solubility space in a structured rather than random fashion and therefore statistical comparison might not be valid. Conversely, two thirds of the tested drugs behave in a manner that is consistent with published paradigms. The current solubility results match literature data (Figure 14) where available indicating that the DoE approaches are investigating a relevant solubility zone, but there are no equivalent large literature data sets available for statistical comparison. Almost all published solubility studies in either human intestinal fluids (Augustijns et al. 2014; Clarysse et al. 2009; Kleberg, F. Jacobsen, et al. 2010) or simulated intestinal fluids (Clarysse et al. 2011; Fuchs & Dressman 2014; Ilardia-Arana et al. 2006; Khadra et al. 2015; Zhou et al. 2017b) indicate that there are drug dependent variations in solubility over and above those induced by variations in media composition. In combination with the results in this study this indicates that a substantial

proportion, around one third, of drugs most probably basic or neutral compounds, will exhibit behaviour at the extremes of current literature based patterns.

2.3.3. Solubility influence of individual DoE factors

For each DoE experiment the software calculates an individual factor's standardised effect on the magnitude and direction of the measured equilibrium solubility, allowing a comparison between factors and drugs. For each drug, statistically significant standardised effect values in the full range study are presented in Figure 18 along with the standardised effect value for that factor in the published fasted and fed studies (Khadra et al. 2015; Zhou et al. 2017b). It must be noted that for each drug only the significant factor effects are presented in Figure 18. The effects of the full range media factors on the drugs are complex because each drug displays a unique profile a result that is similar to the previous fasted (Khadra et al. 2015) and fed (Zhou et al. 2017b) studies. The media components showing the lowest effect on the solubility are buffer and monoglyceride (0 significant results from 9 drugs) followed by salt and lecithin (1 significant from 9), whilst the factors with the biggest influence are pH, bile salt (6 significant results from 9) and sodium oleate (7 from 9). This is comparable to the fasted and fed state DoE where bile salt, pH and sodium oleate were the dominant significant factors but contrasting for lecithin which was also significant in these studies. However, the amplitude of the effect differs between groups and individual drugs confirming the complexity of the interplay between each drug and the system, a feature higlighted in both previous DoE studies. The means of the absolute standardised effect values grouped for acidic, basic and neutral drugs are presented in Figure 19. This provides information on the overall magnitude of a factor influence but masks the direction of the effect. For the three groups of drug pH, sodium oleate and bile salt have a statistically significant influence on solubility in the full range study which is in accordance with the previous reported experiments (Khadra et al. 2015; Zhou et al. 2017b).

For acidic compounds (Figure 18a–b) pH is the most significant factor, which is identical to the two previously reported DoE studies and has already been described for acidic compounds (Clarysse et al. 2009). The direction of effect is comparable (positive) but the magnitude is lower when compared to the published fasted study and similar to the fed study. Sodium oleate and bile salt are the second most significant factors with a positive direction of effect, which is in agreement with the published fasted and the fed state. On the contrary buffer had no influence even though it was significant for the two compounds in the fasted study with a positive effect for phenytoin and a negative effect for indomethacin. The influence of the remaining factors (lecithin, salt and monoglyceride) is also not significant.

For basic compounds (Figure 18c–f) sodium oleate, pH and bile salt are the predominant factors but this effect is variable between drugs. Aprepitant, carvedilol and tadalafil are positively affected by sodium oleate whilst bile salt only positively affects tadalafil and zafirlukast. The influence of pH is not as important as for acidic drugs with a significant effect featured for aprepitant, carvedilol and zafirlukast but not tadalafil. An enhanced solubility is coherent with an increase of pH from 5 to 7 for the weak base zafirlukast. Its pKa value of 4.3 is influencing drug ionisation but for aprepitant (pKa=9.7) this solubility change has to arise via another mechanism since it is un-ionised between pH 5 and 7, for instance with a change in the ionisation of a surfactant. Surprisingly lecithin was not significant which is at variance from the published DoE where sodium oleate, bile salt, pH and lecithin were significant for basic drugs (Khadra et al. 2015; Zhou et al. 2017b).

For neutral compounds (Figure 18g-h) only felodipine and fenofibrate were significantly affected by any factors. Sodium oleate and bile salt had a positive effect on both drugs whilst pH and lecithin only affected felodipine in both cases in agreement with both studies in fasted and fed states. On the other hand probucol was not significantly influenced by any of the factors although previously oleate, bile salt, pH, lecithin, salt and monoglyceride were detected as significant. The influence of pH cannot change the drug ionisation therefore for these drugs the solubility influence has to be associated with a change in ionisation of the media components as presented previously (Khadra et al. 2015; Pedersen et al. 1999; Zhou et al. 2017b). Finally buffer, salt and monoglycerides showed a very small influence on solubility with very low magnitude mostly below the significant level, reflecting the fasted study but contrasting with the fed study where almost all the components were significant.

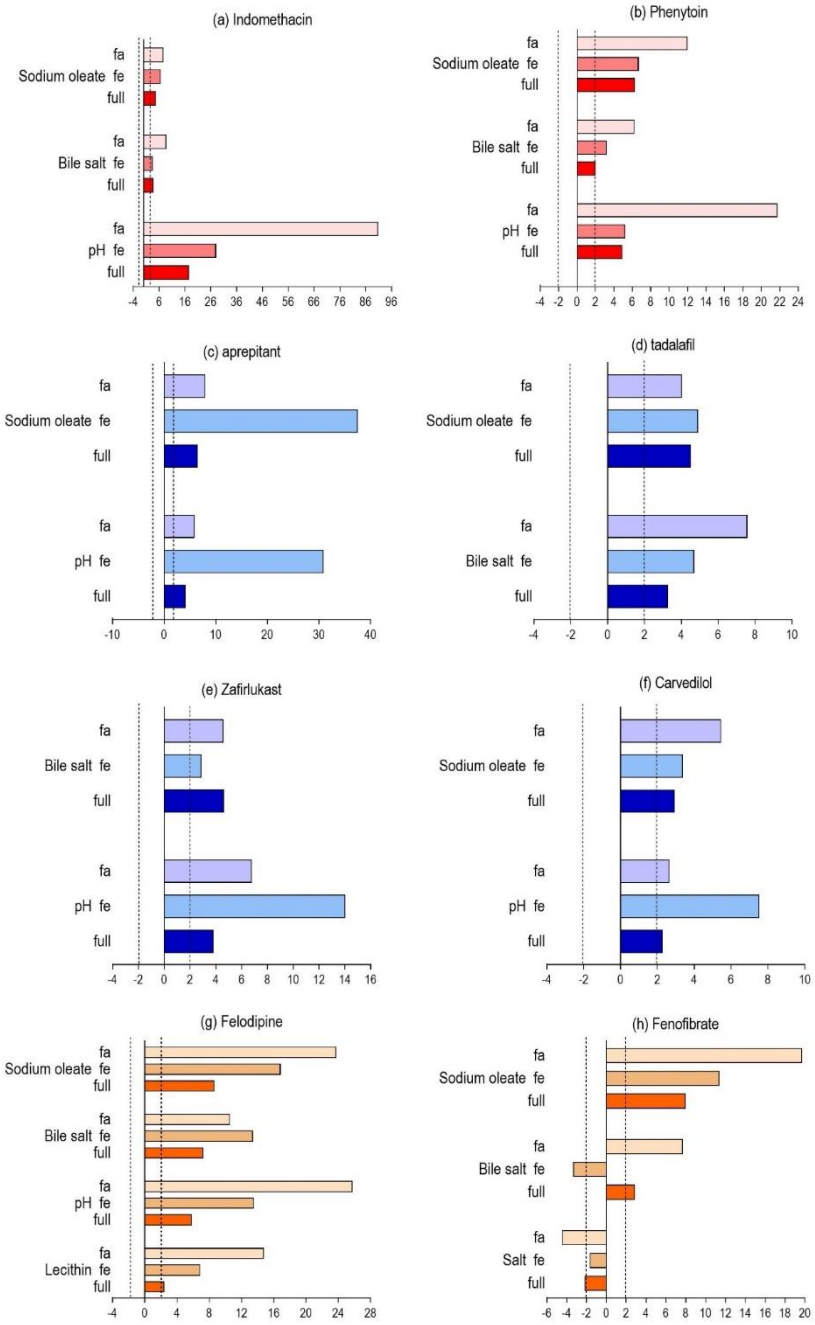

Figure 18 - Statistically significant standardised effect values for individual DoE factors on equilibrium solubility.

Legend: DoE standardised effect values for individual factors (as listed in figure y-axis) on equilibrium solubility. Vertical black lines indicate statistical significance ($p < 0.05$), horizontal bar direction indicates direction of effect, to the right of 0 on axis is positive effect on solubility, bar length indicates the magnitude of the effect. Full: value from current study, Fa: fasted data from (Khadra et al. 2015), Fe: fed data from (Zhou et al. 2017b). NB For each drug non-statistically significant factor effects in this study are not presented.

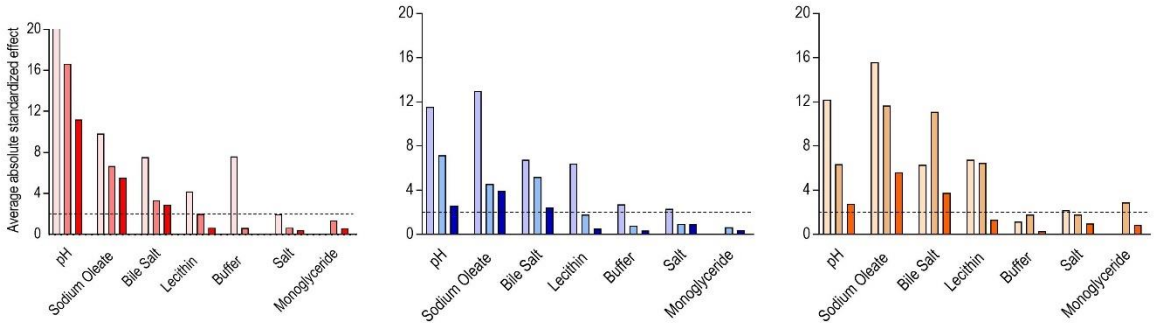

Figure 19 - Average absolute standardised effect values for individual DoE factors on equilibrium solubility.

Legend: Average absolute (NB this removes direction of effect information) standardised effect values for individual factors on equilibrium solubility grouped by drug category. Horizontal black line indicates statistical significance ($p < 0.05$). Acidic Drugs; ■ current full range study, ■ fasted design of experiment solubility data (Khadra et al. 2015); ■ fed design of experiment solubility data (Zhou et al. 2017b). Basic Drugs; ■ current full range study, ■ fasted design of experiment solubility data (Khadra et al. 2015); ■ fed design of experiment solubility data (Zhou et al. 2017b). Neutral Drugs; ■ current full range study, ■ fasted design of experiment solubility data (Khadra et al. 2015); ■ fed design of experiment solubility data (Zhou et al. 2017b)

2.3.4. Solubility influence of DoE factor interactions

The experiment consisted of seven factors and a possible twenty one interactions between the factors per drug. Only 2-way interactions were considered. Three confounded interactions are present, sodium oleate and salt with buffer and monoglyceride, sodium oleate and buffer with salt and monoglyceride, sodium oleate and monoglyceride with salt and buffer. For each drug, statistically significant standardised effect values for factor interactions in the full range study are presented in Figure 20 along with the standardised effect value for that factor interaction in the published fasted and fed studies (Khadra et al. 2015; Zhou et al. 2017b). It must be noted that for each drug non-statistically significant factor interactions in this study are not presented which does not mean that a statistically significant effect was not determined in either the fasted or fed study. Among all the possible factor combinations in this full range DoE a statistically significant effect was present eighteen times which represents approximately 10% of the possibilities. For neutral and acidic drugs eight and seven significant interactions featured respectively while for basic drugs only three. This contrasts

with the fasted and fed DoE where respectively one third and one fifth of the interactions were significant.

For acidic drugs (Figure 20a–b) the effect of factor interactions is consistent within the group, bile salt or sodium oleate are associated in each significant interaction and pH is only present in three of them. The limited effect of pH is surprising since the pKa values of the two acidic factors (oleate and bile salt) is approximately 5 (Holm et al. 2013) and the DoE range is 5–7 which must induce variation in factor ionisation. Interestingly the combination of salt and monoglyceride is significant for both compounds, which could be a confounded effect since this interaction is linked with sodium oleate and buffer.

For basic compounds (Figure 20c–f) only three significant interactions were highlighted and they all include pH with either bile salt or sodium oleate. This is expected as these factors were predominant in the single factor analysis and also reported during the fasted and fed DoE.

For neutral drugs (Figure 20g–i) all the significant interactions are associated with bile salt or sodium oleate, although the oleate and salt interaction for fenofibrate is confounded with buffer and monoglyceride. The positive effect of surfactant has been previously reported in the fasted and fed DoE for this group of drugs.

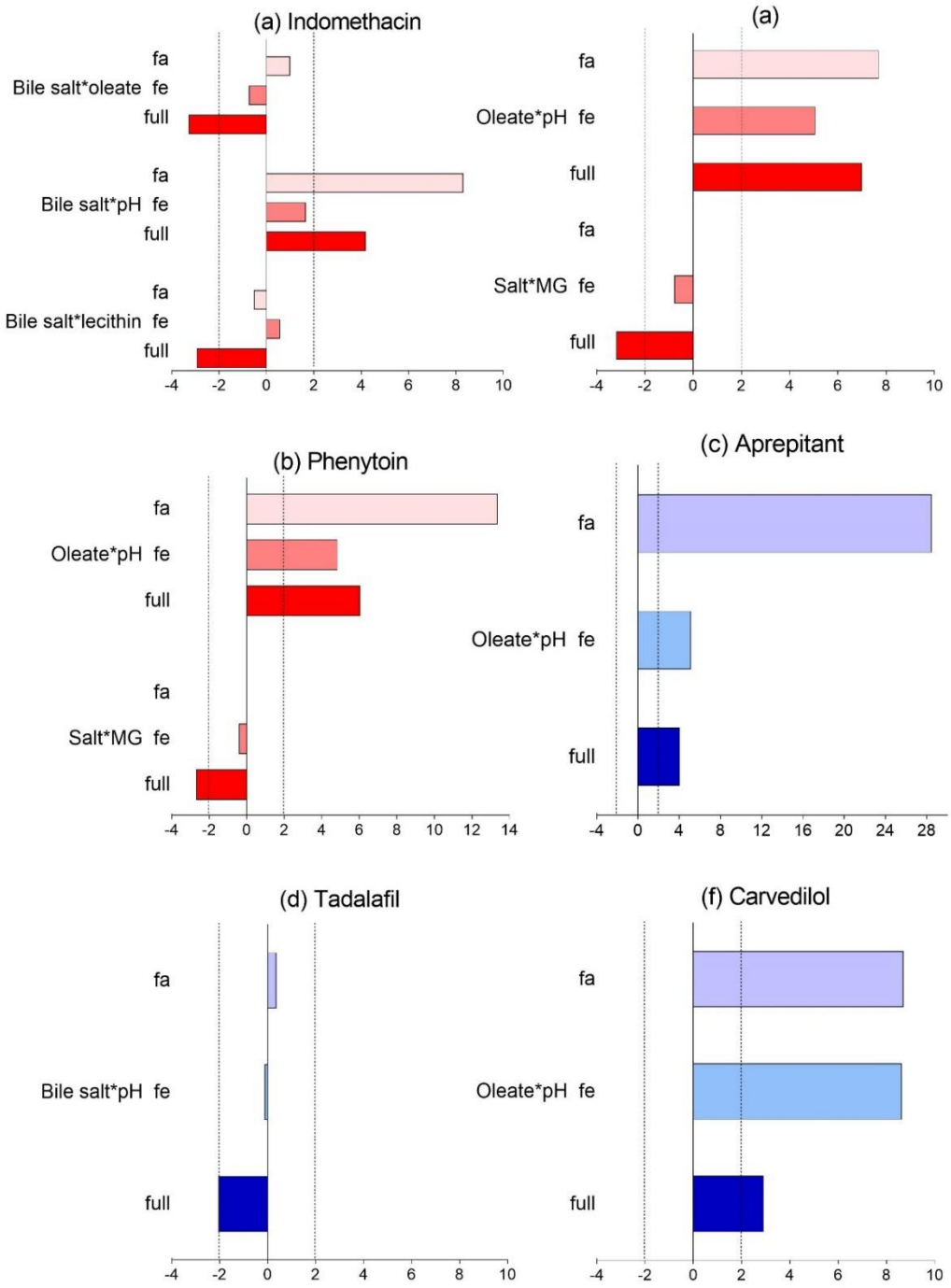

Figure 20 - Standardised effect values for DoE factor interactions on equilibrium solubility. Legend: DoE standardised effect values (x-axis) for interactions between factors (as listed in figure titles) on equilibrium solubility in the fasted (fa), fed (fe) and full range (full) experiment. Vertical dashed black lines indicate statistical significance ($p < 0.05$), bar direction indicates direction of effect, to the right of 0 on x axis is positive effect on solubility, bar length indicates the magnitude of the effect. Full: value from current study, Fa: fasted data from (Khadra et al. 2015), Fe: fed data from (Zhou et al. 2017b). NB For each drug non-statistically significant factor interactions in this study are not presented

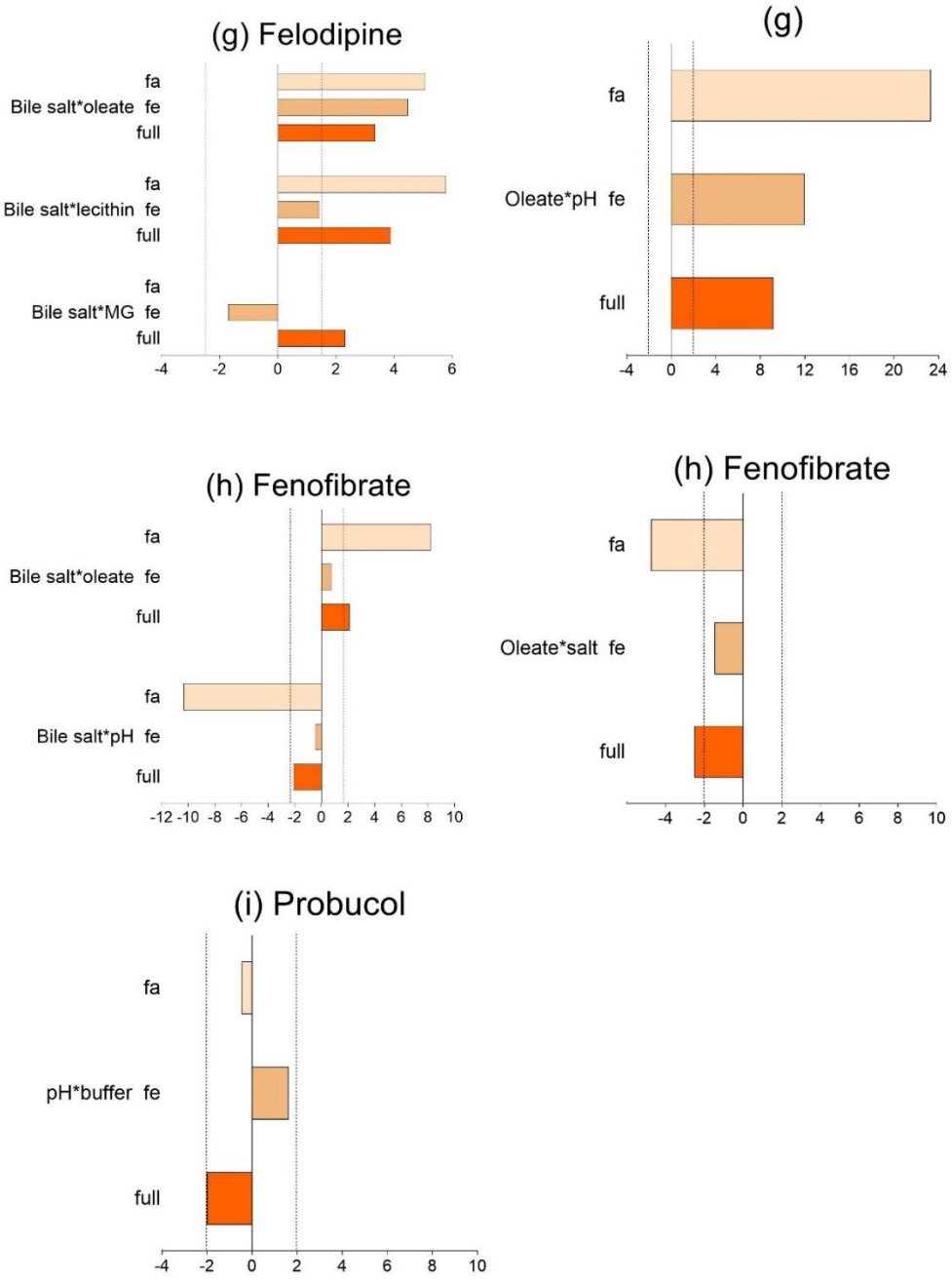

Figure 20 - (Continued)

2.3.5. Statistically significant solubility factor and factor interactions

The means of the absolute effect value of all statistically significant factor and factor interactions arranged by drug group are presented in Figure 21 in order to summarise the full range experimental results. When comparing factor interactions between this full range and published results conclusions should be made with prudence since the fed study employed a different statistical design of experiment (Zhou et al. 2017b). For the acidic drugs pH is not

surprisingly the principal factor. In the published fasted (Khadra et al. 2015) and fed (Zhou et al. 2017b) study pH is involved in every statistically significant combination with either sodium oleate, bile salt or buffer. These interactions are confirmed in the full range experiment as pH, sodium oleate and bile salt are responsible for three out of four. The significant interaction of salt with monolgyceride is a result that is not present in the published fed DoE (Zhou et al. 2017b) but is a confounded interaction in this study with sodium oleate with buffer, it is therefore likely that this is due to a dominant effect arising from sodium oleate. For the basic drugs sodium oleate, pH, bile salt and the interaction between sodium oleate and pH were found to be statistically significant. Interestingly those components are involved in the two significant interactions highlighted in the fed DoE (pH*sodium oleate and lecithin*sodium oleate) (Zhou et al. 2017b). However, in the fasted DoE six different interactions were significant, pH with sodium oleate, salt and lecithin, bile salt with sodium oleate and buffer and then lecithin with salt. For the neutral drugs the previous fasted and fed studies had described a more complex pattern with eight and fifteen significant interactions respectively. Surprisingly the full range experiment is not reflecting this result since only two significant factors were present (sodium oleate with pH and bile salt). The reduced experiment full range DoE is therefore picking up fewer significant factors and factor interactions than the larger focussed experimental studies.

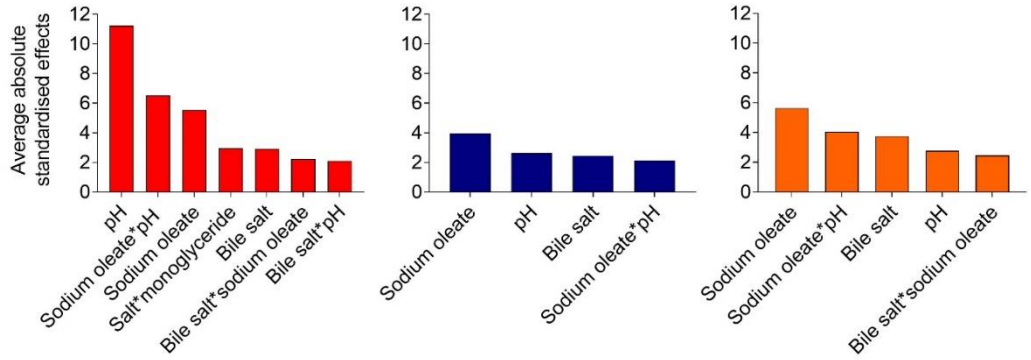

Figure 21 - Average significant absolute standardised effect values for individual factors and factor interactions on equilibrium solubility grouped by drug category. Red coloured bars for acids, blue for basics and orange for neutrals. NB Only statistically significant results are presented

2.3.6. Comparison of full range DoE with published fasted and fed DoE

For each compound the significance of individual media factors on the equilibrium solubility is presented in Table 12 juxtaposed to the published fasted and fed DoE results. The factor

least consistent with previous studies is lecithin (2 matches from 9 drugs) followed by buffer (3 matches from 9 drugs), salt, monoglyceride and bile salt are intermediate (4 or 5 matches from 9 drugs) with pH (6 matches from 9 drugs) and oleate (7 matches from 9 drugs) the most consistent. In addition for any factor the full range study has the lowest number of significant findings when compared to the published studies, a result also applicable to two-way factor interactions. The difference in the ability to detect the significance of a factor's contribution to equilibrium solubility may be due to a number of differences between the studies. The reduced number of sample points for the full range (32 vs 66 (fasted) or 92 (fed)) study must reduce the statistical resolution and therefore only factors which are highly significant or not significant are detected correctly, see Figure 19 and Table 12. In addition a design of experiment statistically combines high and low levels of a factor to construct the measurement points, covering the full range (fasted to fed) will produce factor ratios that are not likely to be biorelevant (Riethorst et al. 2015). This may be why lecithin has the lowest consistency since the influence of lecithin observed in the previous published DoEs (Khadra et al. 2015; Zhou et al. 2017b) was the least significant therefore it is not reproduced or captured in this full range study. The importance of the “solubilising” capacity (combination of bile salt, lecithin, and sodium oleate) has been reported to significantly enhance solubility (Kleberg, F. Jacobsen, et al. 2010; Pedersen et al. 2000; Söderlind et al. 2010) but this is not evident in this study. Further more detailed studies with increased drug numbers and properties along with scaled experimental number design of experiment approaches would be required to fully elucidate the reasons for these findings.

Table 12 Comparison of the statistical significance of DoE factors

	Factor																				
Drug	oleate			Bile salt			pH			lecithin			buffer			salt			Monoglyceride*		
	fstd	fed	Full	fstd	fed	Full	fstd	fed	Full	fstd	fed	Full	fstd	fed	Full	fstd	fed	Full	fstd	fed	Full
Phenytoin	S	S	S	S	S	NS	S	S	S	S	NS	NS	S	NS	NS	S	NS	NS	-	S	NS
Indomethacin	S	S	S	S	S	S	S	S	S	NS	NS	NS	S	NS	NS	NS	NS	NS	-	NS	NS
Aprepitant	S	S	S	S	S	NS	S	S	S	NS	S	NS	NS	S	NS	NS	S	NS	-	NS	NS
Tadalafil	S	S	S	S	S	S	S	NS	NS	S	NS	NS	S	NS	NS	S	NS	NS	-	NS	NS
Zafirlukast	S	NS	NS	S	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	-	NS	NS
Carvedilol	S	S	S	S	S	NS	S	S	S	S	NS	NS	NS	S	NS	NS	NS	NS	-	S	NS
Felodipine	S	S	S	S	S	S	S	S	S	S	S	S	NS	NS	NS	NS	NS	NS	-	NS	NS
Fenofibrate	S	S	S	S	S	S	S	NS	NS	S	S	NS	S	S	NS	S	NS	S	-	S	NS
Probucol	S	S	NS	NS	S	NS	S		NS	NS	S	NS	NS	NS	NS	NS	S	NS	-	S	NS
Total significant	9	8	7	8	9	5	9	7	6	6	4	1	4	3	0	3	2	1	-	4	0

Fstd = fasted state media design of experiment (Khadra et al. 2015)

Fed = fed state media design of experiment (Zhou et al. 2017b)

Full = full range media design of experiment

*Monoglyceride not included in the fasted state media design of experiment

S = factor statistically significant in design of experiment study

NS = factor not statistically significant in design of experiment study

Shaded box = no consistent result between studies

2.4. Conclusion

The objective of this study was to determine the feasibility of combining the previous fasted and fed DoE into one smaller full range experiment in order to obtain comparable results regarding the influence of gastrointestinal media components on the equilibrium solubility of BCS class II drugs. This full range DoE produced interesting results regarding the general solubility space, which overall is comparable to published fasted (Khadra et al. 2015), fed (Zhou et al. 2017b), simulated and human intestinal fluid equilibrium solubility values (Augustijns et al. 2014). A statistical comparison of the published fasted and fed solubility distributions indicates that these are significantly different, with the fed higher than the fasted a result which is in agreement with literature data based on single measurements (Augustijns et al. 2014). A statistical comparison of the full range measured equilibrium solubility values with a combination of the published fasted and fed indicates that this full range experiment was statistically equivalent to the previous fasted and fed DoE for six out of the nine drugs tested (Figure 15 - 17). However, for three drugs statistically significant differences were detected indicating that for these drugs the full range DoE will not provide equivalent equilibrium solubility information to separate larger studies. It is likely that this behaviour will be present in other drugs which will be most probably either basic or neutral in character, however further research is required to fully elucidate the molecular properties that produce this effect. The measured solubility distributions for each drug in the full range experiment and the published fasted and fed experiments were non-normal a result that may be due to the structured sampling induced by the DoE, the fact that the distribution is non-normal either through the presence of multiple distributions or extreme points or that the number of data points is not sufficient to sample the distribution. Further studies will be required to determine the origin of this result however it implies that a single solubility measurement without knowledge of the solubility distribution will be of limited value. Overall the three drug groups exhibited a similar profile with respect to the most significant factors and two-way factor interactions controlling solubility when compared to the published fasted and fed studies. For acidic drugs unsurprisingly pH and oleate were dominant (Khadra et al. 2015; Zhou et al. 2017b) with bile salt also significant. For the neutral and basic drugs three factors pH, bile salt and sodium oleate were dominant along with a two-way interaction of sodium oleate with pH and for neutral drugs only bile salt with sodium oleate. Although there was variation between the drugs the four other factors (lecithin, monoglyceride, salt and buffer) were on average not significant at all along with around 90% of possible two-way factor interactions. The reduced incidence of significant effects with individual factors and two-way factor interactions may be a consequence of the reduced number of measurement points within the design of experiment and or the combination of factor values covering the fasted and fed range

leading to systems that are not biorelevant. Notably, lecithin did not significantly influence solubility a result that contrasts with previous published information as it is reported to be essential in the solubilising capacity of simulated intestinal fluids (Söderlind et al. 2010) and was significant in published DoE studies (Khadra et al. 2015; Zhou et al. 2017b). The reduced experiment full range DoE study therefore provides equilibrium solubility values that for the majority of drugs will be equivalent to larger studies but with a lower statistical ability to identify the significant factors and factor interactions that influence solubility. Further statistical refinement might be possible to tease out the differences between the fasted and fed states using for example a dual small scale DoE covering both states. This might also provide information to determine why some drugs do not produce equivalent solubility results between DoE approaches.

Chapter 3

Application of the DCS classification to assist formulation development

3. Application of the DCS classification to assist formulation development

3.1. Introduction

In the previous chapter it has been shown that *in vitro* biorelevant solubility was more pertinent to anticipate the *in vivo* variability in solubility. This is why a biorelevant medium was developed and applied to drugs with various ionisation properties. The results demonstrated consistency with the previous studies as well as with the published solubility data in fasted and fed simulated or human intestinal fluids. These results are particularly important for class II drugs defined as poorly soluble and highly permeable according to the biopharmaceutic classification system (BCS). In this system solubility definition is based on the minimum solubility observed in buffers of 250ml over a physiological pH range (usually from 1.2 to 6.8). Yet for most of the drugs this method is likely to underestimate the solubility observed *in vivo* since it has been reported that the presence of physiological gastro-intestinal fluid secretions and food intake can largely influence and increase solubilisation. The BCS classification was acknowledged as a regulatory classification which in the interest of patients legitimately adopted relatively conservative positions on the definition of limited solubility, permeability and dissolution rate (Amidon et al. 1995). As stated by Amidon and coworkers those three parameters are considered to be the key factors controlling the *in vivo* absorption and performance of immediate release oral dosage forms. The introduction of the BCS classification enabled biowaivers of *in vivo* bioequivalence studies for BCS I drugs which led to significant changes in the development of oral dosage forms. Nevertheless with the increasing use of more realistic methods to estimate *in vivo* solubility and dissolution such as the introduction of biorelevant dissolution media an updated classification was suggested (Butler & Dressman 2010). This revised version of the original BCS was designed to more accurately classify drugs with the use of better estimates of solubility and a more sensible fluid volume in the GI tract.

It was designed not as a simple regulatory classification but as more appropriate to help formulation development of oral drug products. The incorporation of an estimate of fasted intestinal solubility along with the dose/solubility ratio provides a more relevant classification of oral products and more information on the factors limiting oral absorption. According to this new classification a new level of distinction is available for BCS II drugs and allows the identification of dissolution-rate limited drugs (DCS class II-a) and solubility limited drugs (DCS II-b). This distinction between dissolution-rate limited *versus* solubility-limited simplifies the choice of the appropriate formulation and provides a very useful tool for formulators in the early stage of development. With the increasing number of solubilising techniques developed in the recent years such as the particle-size reduction, the amorphous solid dispersion, the nanosuspensions and the lipid-based formulations, it is particularly interesting to anticipate

the formulation choice of BCS II drugs. In this study the DCS method was applied on the nine drugs investigated in chapter 2 along with three cancer molecules developed by the Cancer Research UK (CRUK).

3.2. Objective

The DCS integrates more realistic solubility estimates therefore the objective of this work was to better identify the drugs as DCS class II-a or class II-b in order to anticipate the *in vivo* oral absorption. This new classification is a convenient way of assessing developability issues by choosing the right formulation depending on whether dissolution rate, solubility or permeability would be defining *in vivo* oral absorption. The first objective was to compare the observed DCS category for each drug between the FaSSIF solubility and the full DoE approach solubilities in order to analyse the impact of variable solubility values on the resulted DCS classification. A posteriori verification could be done since for the Orbito drugs the formulations and pharmacokinetic data are already published in the literature. The second objective was to investigate three cancer drugs in the process of development to anticipate their *in vivo* behaviour in terms of solubility, dissolution and absorption. Furthermore the possibility of using the extreme biorelevant media conditions (media factors at high or low concentration levels) was assessed to check whether they could capture the extent of variability with only two points.

3.3. Materials and methods

The nine Orbito drugs were investigated along with three CRUK molecules in development. The physico-chemical caracteristics and maximum oral dose observed in the clinic are listed in the following table (Table 13). For each drug the dose/solubility ratio was calculated with the estimated intestinal solubility in FaSSIF medium and plotted with the corresponding effective permeability. The DCS category of each drug was depicted on the DCS graph. Then the estimated biorelevant solubilities using a DoE approach (*cf.* chapter 2) were also used to calculate the dose/solubility ratio and all the individual data points were plotted on the DCS graph to study the effect of the variability generated by the DoE. In addition the two extreme media conditions, represented by the measured solubility where all the media factors are at their high or low concentration levels, were highlighted and compared to the single FaSSIF medium to inform on the potential utility of additional solubility information.

Table 13 Physicochemical properties of the nine studied drugs and their maximum oral dose observed in clinic

Drug	pKa	MW	Log P	P_{eff}	FaSSIF	Max dose
Indomethacin	4.5 (a)	357.7	4.2	7.49	0.37[d]	100
Phenytoin	8.3 (a)	252.2	2.4	8.37	0.0058[c]	300
Carvedilol	7.8 (b)	406.4	4.2	6.80	0.136[e]	50
Tadalafil	0.8 (b)	389.4	1.7	7.3	0.01[f]	20
Zafirlukast	4.3 (b)	575.6	5.4	6.42	0.0024[c]	20
Aprepitant	2.8 (b) / 9.7 (a)	534.4	4.5	7.06	0.023[e]	125
Felodipine	Not ionised	384.2	3.8	7.62	0.053[e]	10
Fenofibrate	Not ionised	360.8	5.2	7.61	0.0096[d]	200
Probucol	Not ionised	516.8	10.6	6.46	0.0034[e]	500
AT13148	1.64(a) 8.57(b) 11.79(a)	386.7	2.1	0.76[i]	0.0023[g]	300
Drug X	na	572.4	na	0.27[i]	na	100[h]
AZD424	na	529	na	3.2[i]	na	100[h]

All data from Pubchem otherwise mentioned

Molecular weight in g/mol (MW), lipophilicity octanol/water (logP), all effective permeability at pH 6.5 (unit 10^{-4}cm/s) predicted with ACD/I-Lab (Advanced Chemistry Development, Inc.) otherwise specified, fasted state simulated intestinal fluid (FaSSIF) in mg/ml, max dose in mg, (a) for acids, (b) for bases, na = not available

[C] (Fagerberg et al. 2015)

[D] (Clarysse et al. 2011)

[E] (Söderlind et al. 2010)

[F] experimentally determined

[G] mean experimental value (N=3)

[H] hypothetical maximum dose

[I] Predicted using Simcyp simulator

3.4. Results and discussion

3.4.1. Application of the DCS to the studied drugs

The classification of the nine orbito drugs according to the Developability Classification System is presented in Figure 22. Although the nine drugs were previously classified as poorly soluble (class II) by the BCS classification, three drugs (felodipine, indomethacin and carvedilol) were upgraded to the DCS class I with the use of a more relevant solubility and intestinal volume. Felodipine did benefit from the biggest biorelevant solubility enhancement. In fact, its calculated dose/solubility ratio with FaSSIF was less than 250 ml which means that this drug would be classified as highly soluble even within the BCS system. Probucol was the least soluble and the use of an estimated FaSSIF solubility did not improve its BCS category. Zafirlukast, fenofibrate, phenytoin and probucol were categorised as DCS class II-b which means that the biorelevant solubility did not influence positively the expected *in vivo* behaviour and thus solubility remains the limiting factor for those drugs (Table 14). Aprepitant and tadalafil fell into the DCS class II-a (Table 14) therefore for those two drugs poor solubility is no longer an issue and could be offset by the high permeability. In other words, complete absorption could still be achieved if formulation of the dosage form is well designed. The three last drugs, felodipine, indomethacin and carbedilol fell into the DCS class I. A significant influence of *in vivo* intestinal fluid solubilisation was observed for these drugs which means that complete absorption is expected in clinic.

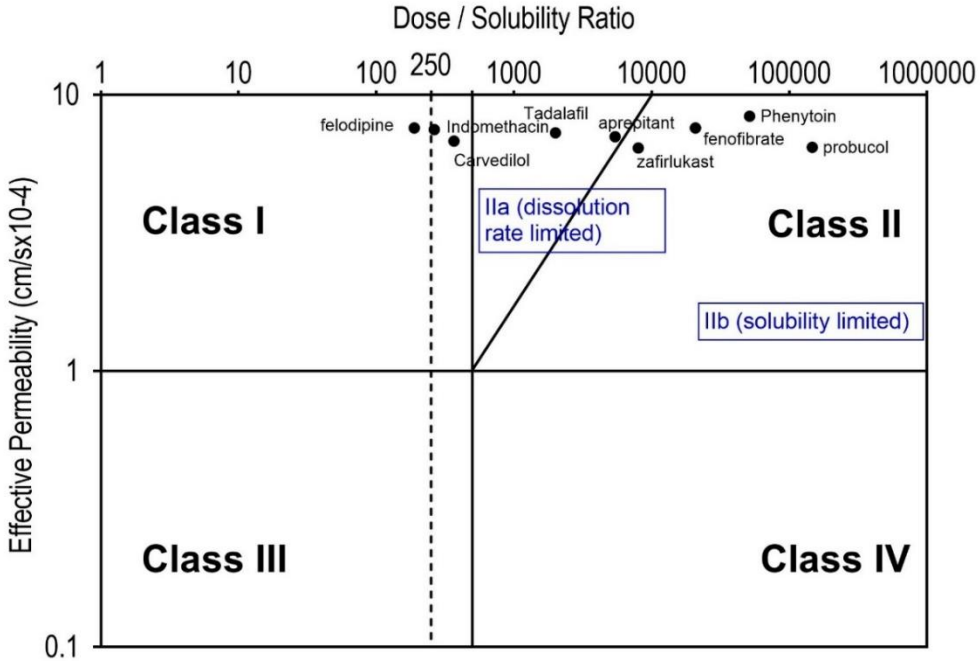

Figure 22 - DCS classification of the nine studied drugs

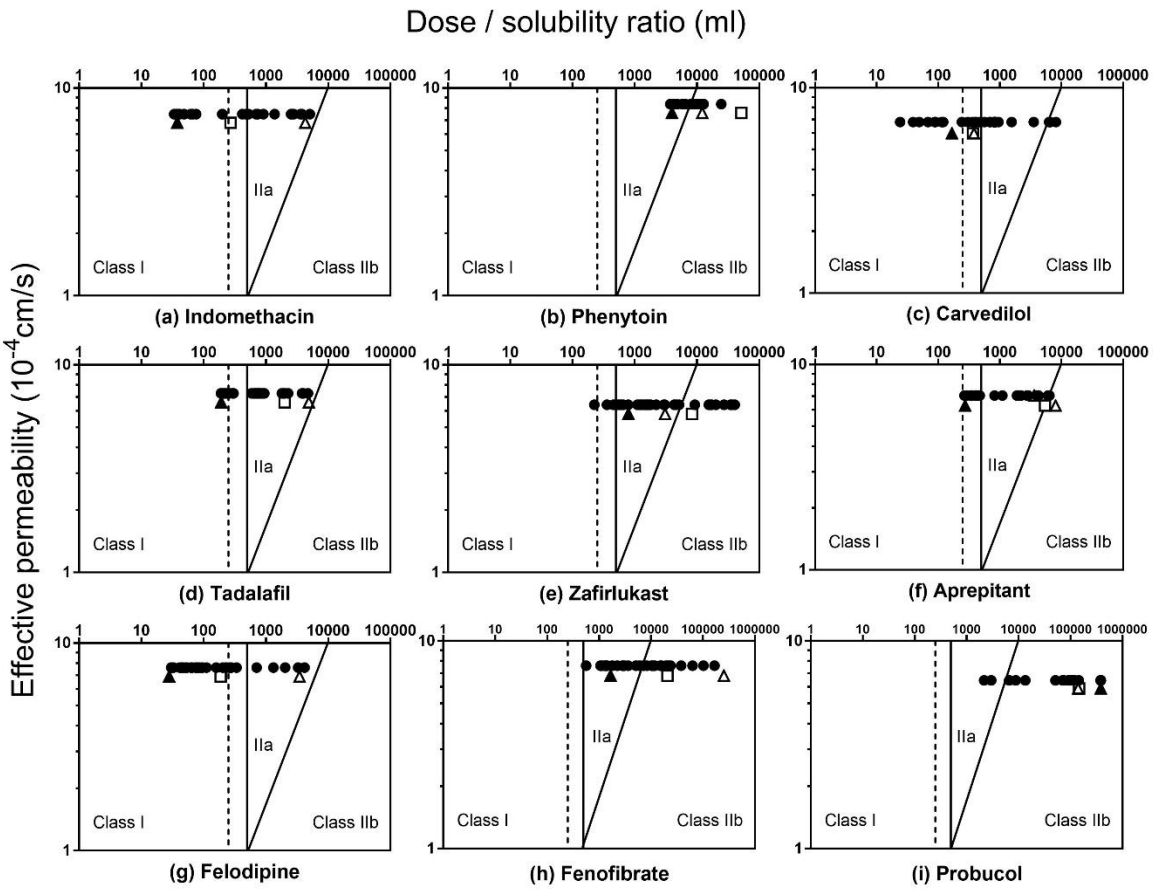

Figure 23 - Application of the DCS classification on the Orbito drugs with the variability of estimated solubility in the DoE biorelevant media simulating the fasted and fed intestinal fluid. The dotted line represents the BCS limit of solubility between BCS class I and II (250 ml). The black dots represent the individual estimated solubility points of the DoE experiment. Δ represents the extreme DoE media conditions where all the factors are at high level (black triangles) or low level (empty triangles) and □ represents the single FaSSIF solubility point. NB: Since all the drugs are highly permeable (P_{eff} > 1x10^{-4} cm/s) the DCS plots are only showing the top classes I and II

The application of the DCS classification with the estimated solubility using the DoE method is illustrated in Figure 23. The variability of solubility was expressed by the 32 different media simulating the fasted and fed intestinal fluid. It is evident that all the drugs benefited from a shift in classes with the influence of the observed variability. The drugs showing the biggest variability are moving from DCS class II-b to class I (carvedilol and zafirlukast) while phenytoin which features the smallest variability is simply moving from class II-b to class II-a (Table 14). Zafirlukast, fenofibrate, phenytoin and probucol drugs were categorised in DCS class II-b when single FaSSIF was used as input but further when the DoE was used phenytoin,

probucol and fenofibrate demonstrated solubility points in class II-a whilst for zafirlukast some data points fell into the DCS class I.

Fenofibrate and probucol are perfect examples of DCS class II-a drugs as regardless of their very highly lipophilic profile (logP = 5.2 and 10.6 respectively) and poor solubility they can benefit from an enhancement of oral absorption by an appropriate formulation. The intestinal absorption of probucol has been reported as very low (2 - 8%) (Zaitseva et al. 1995) however a nanosizing particulation technique was able to significantly improve its *in vitro* dissolution and *in vivo* absorption (Tanaka et al. 2012). For fenofibrate the reported bioavailability of 60% with a large inter-individual variability is an issue (Miller & Spence 1998) yet its bioavailability has also been largely improved by a nanosizing particle size reduction technique (Choi et al. 2015). For the drug phenytoin it has been reported that the bioavailability of this drug was influenced by the particle size (Richens 1979) which is a specific characteristic of class II-a drugs.

On the other hand the pharmacokinetics of zafirlukast has showed that the drug is rapidly absorbed however it is subject to an important hepatic first-pass metabolism (Dekhuijzen & Koopmans 2002). This study confirms the expected good *in vivo* absorption of this drug according to its observed DCS classification.

Aprepitant and tadalafil displayed the same pattern in both cases since they were in the DCS class II-a with the FaSSIF solubility and exhibited points in the DCS class I with the DoE solubility values. As a consequence the poor solubility of these two drugs is not expected to be problematic and this was confirmed *in vivo* since tadalafil is rapidly absorbed with a bioavailability of 80% (Francis & Corbin 2003) while aprepitant also demonstrates a good oral bioavailability of 60 to 65% (Majumdar et al. 2006).

Felodipine, indomethacin and carvedilol were already into the DCS class I with an assumed high estimated solubility in FaSSIF and a high permeability therefore the DoE confirmed the expected favourable *in vivo* behaviour of those drugs. Nevertheless the important variability observed for carvedilol with the DoE approach showed that a few data points were falling into class II-b but with no consequences. Indeed it has been demonstrated that the pharmacokinetics of carvedilol exhibit a rapid absorption but suffers from an important first-pass metabolism in the liver (Morgan 1994). Similarly for felodipine its *in vivo* absorption is rapid and complete following oral administration in the clinic but an extensive first-pass metabolism yields a low bioavailability of 15% (Dunselman & Edgar 1991). The *in vivo* absorption of indomethacin is also rapid and complete but with important inter- and intra-individual variabilities (Helleberg 1981).

The estimated solubility at the extreme biorelevant media conditions for the Orbito drugs (all high or all low surfactant concentration) are also illustrated on Figure 23. Logically when the

concentration of all the factors included in the DoE media were high, the observed biorelevant solubility was expected to reach a maximum value. Conversely when all the factors were at the low level the solubility was expected to be minimum. Interestingly this was the case for four drugs (indomethacin, tadalafil, felodipine and aprepitant) where the extreme media conditions were able to capture the extent of variability. However for the other drugs the high and low extreme conditions were only capturing a fraction of the extent of variability (Figure 23).

For fenofibrate 67% of the solubility points were covered by the extreme values yet it resulted in the same DCS classification since the two values were still covering the classes II-b and II-a. Similarly for phenytoin the majority of the solubility points were covered (86%) which also resulted in the same DCS classification. On the other hand for carvedilol and zafirlukast only 37% and 39% of the DoE variability were captured respectively. As a consequence if one would be to measure solubility only in these three media the result could be misleading when applying the DCS system given that for carvedilol the two data points were in the DCS Class I while for zafirlukast they were in the DCS class II-a. Probucol showed the least suitable profile because only 23% of the data points were included within the minimum and maximum simulated media conditions. This outcome resulted in the underestimation of classification of this drug (DCS class II-b) while the DoE solubility predicted a shift into the DCS class II-a.

For each drug the estimated FaSSIF solubility is also highlighted to allow comparison (empty square) (Figure 23). Interestingly for all the drugs the single FaSSIF value was contained within the same solubility space as the DoE experiments except for phenytoin where the observed DoE solubilities were greater. This outcome means that the DoE is relevant as it is scanning the same solubility space as the FaSSIF solubility as already reported in the chapter 2. In addition it can be noted that using a single FaSSIF provided a good estimation of the *in vivo* solubility. Nevertheless the complex dynamic environment of the intestinal tract influenced by changes in pH and surfactant concentrations gives more relevance to the DoE especially for ionised drugs. The application of the DoE study accentuated the pertinency of conducting various media conditions when forecasting the biorelevant solubility of drugs. The drug specificity behaviour has been emphasised in the previous studies (Khadra et al. 2015; Zhou et al. 2017b) and it was highlighted once more since no evident pattern was found among the nine drugs. Indeed the estimated solubilities were not consistently distributed with the two extremes at the end and the single FaSSIF in the middle (Figure 23).

3.4.2. Application of the DCS to three CRUK drugs

The application of the DCS classification with the estimated solubility using a DoE method applied to three CRUK drugs is illustrated in Figure 24. According to their physicochemical

properties (poor permeability and poor solubility) AT 13148 and drug X (Figure 24a and b) were classified as BCS IV which is the worst case scenario for drugs intended to be administered orally. The estimated solubility of AT 13148 in FaSSIF media (Figure 24a) did not significantly improve its dose/solubility ratio therefore the it was categorised as a DCS IV and as a consequence the *in vivo* absorption and bioavailalbility of this molecule is expected to be problematic with inconsistent pharmacokinetics.

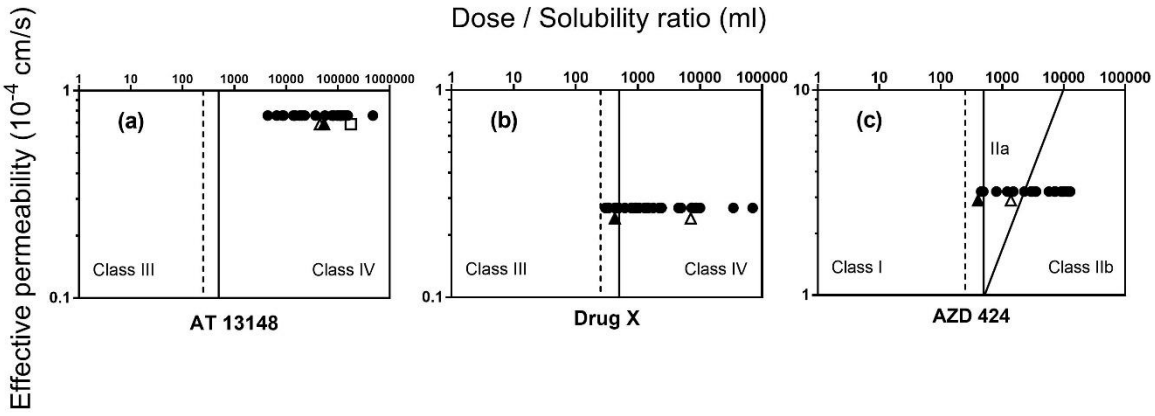

Figure 24 - Application of the DCS classification on the CRUK drugs with the variability of estimated solubility in a biorelevant media simulating the fasted and fed intestinal fluid. The dotted lines represent the BCS limit of solubility between BCS class I and II (250 ml). The black dots represent the individual estimated solubility points in the DoE biorelevant media. Δ represent the extreme DoE media conditions where the concentration of all the factors are at high (black triangles) or low level (empty triangles) and □ represents the single FaSSIF solubility point. NB: the drugs AT 13148 and drug X are poorly permeable (Peff < 1x10-4 cm/s) therefore only the DCS bottom classes III and IV are plotted whereas for AZD 424 the DCS plot is showing the top classes I and II since the permeability is high.

For drug X (Figure 24b) the estimated solubility in FaSSIF media was not determined but the observed DoE solubilities demonstrated a shift into the class III of the DCS. Molecules identified between the DCS class III and IV can be investigated to determine when solubility rather than permeability will become an issue for formulation development. The bioavailability of these drugs will be dose dependent since the dose/solubility ratio will increase with the dose and therefore absorption will be greater at lower doses. It should be noted that for drug X the dose/solubility ratio was estimated with a theoretical dose of 100 mg as a consequence this molecule is expected to show a dose dependent bioavailability. If the maximum therapeutic dose in clinic is lower than 100 mg, then this drug would certainly fall into the DCS class III

and therefore solubility will no longer be an issue. On the other hand if the maximum therapeutic dose in clinic is greater than 100 mg this drug will less likely show an interesting *in vivo* behaviour.

AZD 424 was classified as a BCS class II drug and the influence of the DoE biorelevant media provided an encouraging formulation development considering the shift from the DCS class II-b to the DCS class II-a with three data points falling into class I. Again the theoretical dose of 100 mg means that *in vivo* absorption and bioavailability will certainly depend on the therapeutic dose expected in clinic. In addition given the predicted DCS classification (class II) this drug will be a perfect candidate for a specific solubility enhancing formulation such as particle size reduction or an amorphous solid dispersion for absoption and bioavailability improvement.

The estimated solubility at the extreme media conditions for the CRUK drugs (all high or low surfactant concentration) are illustrated on Figure 24. For AT13148 the two DoE values representing the extreme media conditions were identical yet this had no consequence on the solubility behaviour of the drug since all the estimated solubilities for this drug were in the class IV and therefore the classification remained unchanged. For drug X the extreme media conditions captured only 56% of the solubility points yet they were still able to capture the shift from DCS class IV to DCS class III. For AZD 424 only 15% of the data were captured with the extreme media points and they covered the DCS class II-a to class I. As a consequence by applying this approach of using only two solubility points for this drug the lowest solubility points featured in class II-b would be ignored and the biorelevant solubility would be overestimated.

Table 14 Evolution of the biopharmaceutic classification of the studied drugs

Drug and dose	BCS category	DCS category with fassif	DCS category with DoE
Indomethacin 100 mg	II	I	I / IIa
Phenytoin 300 mg	II	IIb	IIa / IIb
Carvedilol 50 mg	II	I	I / IIa / IIb
Tadalafil 20 mg	II	IIa	I / IIa
Zafirlukast 20 mg	II	IIb	I / IIa / IIb
Aprepitant 125 mg	II	IIa	I / IIa
Felodipine 10 mg	II	I	I / IIa
Fenofibrate 200 mg	II	IIb	IIa / IIb
Probucol 500 mg	II	IIb	IIa / IIb
AT 13148 300 mg	IV	IV	IV
DRUG X 100 mg[a]	IV	na	III / IV
AZD 424 100 mg[a]	II	na	I / IIa / IIb

[a] Theoretical dose

3.5. Discussion

The BCS has been largely acknowledged in the pharmaceutical industry as a regulatory classification yet the introduction of the DCS has demonstrated a much more relevant approach with direct application in the formulation development of drugs to forecast the *in vivo* absorption. Although the revised classification provides a relatively simple and efficient tool to help formulators some limitations must be considered given the following assumptions are made when using the DCS classification. (i) The DCS uses a single estimated solubility and permeability to characterise the absorption in the intestine. Interestingly this has been partly addressed within this study considering the work on the DoE solubility using drugs with various ionisation properties. (ii) The total fluid volume in the GI tract has been set to 500 ml in the fasted state. This is likely to be overestimated in the fasted state since it has been reported that the volume of the stomach before a meal was around 30 ml (Steingoetter et al. 2006) while in the small intestine an average of 100 ml has been found in different studies (L. Marciani *et al.*, 2010 (a); Luca Marciani *et al.*, 2010 (b) ; Placidi *et al.*, 2010). Nevertheless in

the fed state the volume in the small intestine was found to range between 18 to 660 ml and to be highly dependent on the amount and content of food ingested (Luca Marciani et al. 2010). (iii) For poorly soluble drugs the theoretical concept of compensation is assumed and states that high permeability will offset poor solubility (Fagerholm et al. 1999). The use of the DCS is therefore more suitable for non-ionised drugs as already reported (Butler & Dressman 2010) since the ionised drugs will show a large pH-dependent solubility profile which cannot be fully investigated in one single FaSSIF value.

Ideally when developing a new molecule the solubility profile should be investigated in actual human intestinal fluid (HIF) to make informed decisions. However for obvious reasons this method is not applicable in a standard solubility screening test this is why the biorelevant design of experiment study was developed in order to characterise the intestinal solubility of drugs with various ionisation properties with a sensible number of measurements. Yet the design of experiment method remains laborious for larger scale routine assessment of biorelevant solubility in an industrial development setting. Therefore the suggestion of using only three sensible biorelevant media points was studied. Legitimately the fasted state simulated intestinal fluid (FaSSIF) media was included and the two DoE media representing the extreme conditions were considered. However as demonstrated here it only worked for four out of nine drugs. In fact, the study of design of experiment showed that coherent solubility behaviour could be expected for some drugs such as the acidic drugs indomethacin and phenytoin which were mainly driven by pH. Similarly the neutral drugs fenofibrate and felodipine were essentially influenced by the solubilising molecule sodium oleate, bile salt and lecithin. On the contrary the solubility behaviour of the other drugs was more unpredictable and a drug specificity was concluded since no obvious trend could be identified. When applying the DCS classification system with a new poorly soluble drug, ideally for ionised drugs the investigator should determine the full pH-solubility profile when possible otherwise the FaSSIF value should be taken with prudence since it can under or overestimate the biorelevant solubility as seen with the drugs aprepitant and phenytoin. For non-ionised drugs a large solubility enhancement *in vivo* is generally expected therefore a single media could be sufficient.

Furthermore it must be noted that the statistical design of experiment (DoE) study associates maximum and minimum concentration levels of the different intestinal components in order to combine the fasted and fed ranges. As a consequence this generated concentration ratios of factors not truly biorelevant which could explain the unpredictable solubilities observed at the extreme media conditions (Riethorst et al. 2015). A further study would be required to address this point and recommend a refined design of experiment with more physiological ratios.

The estimation of *in vivo* solubility in the small intestine is logical for neutral drugs and weak acids as this is where the majority of the dissolution will occur. On the other hand weak bases are known for being more soluble at lower pH especially for drugs with a pKa between 3 to 9 and also known for precipitating in the small intestine due to supersaturation (Psachoulias et al. 2011). Absorption of weak bases will largely depend on gastric conditions (pH and residence time) therefore factors affecting the variability of absorption such as feeding state, disease, age or other medications may have a greater influence on weak bases since conditions in the stomach are more variable than in the intestine. In practice, two types of dissolution will be observed for weak bases, the one mainly taking place in the stomach (*e.g.* ketoconazole (Van Der Meer et al. 1980)) and the one partly taking place in the stomach and partly in the intestine. For instance dissolution of dipyridamole is increased at low gastric pH but oral absorption is limited by precipitation in the upper intestine (Derendorf et al. 2005). Although various methods have been suggested to study the precipitation of weak bases *in vitro* it remains more challenging than predicting dissolution rates. For drugs that do not precipitate estimates of gastric solubility may be sufficient to predict oral absorption.

3.6. Conclusion

The introduction of the BCS system as a regulatory classification allowed major changes in the development of drugs especially for BCS class I. By exhibiting a favourable profile (highly soluble and highly permeable) the drugs from this class can be exempted from clinical studies when submitting a bioequivalence study. With the new DCS classification the very conservative definitions of high solubility and permeability by the BCS were discussed and new approaches were recommended. The aim of the DCS classification was not to establish a new regulatory guidance but to provide a more realistic structure to inform formulation developers on the expected *in vivo* oral absorption of drugs. This new approach is mostly beneficial for poorly soluble and higly permeable drugs (BCS class II) since the DCS integrates a new discrimination within the class II allowing the identification of dissolution-rate limited drugs (class II-a) and solubility-limited drugs (class II-b). One of the most notable developments was the use of biorelevant solubility (*e.g.* FaSSIF) and the solubility limited absorbable dose (SLAD). These two concepts are very important for BCS II drugs since their poor water-solubility can be largely enhanced *in vivo*. The study presented herein on the application of the DCS concept to various ionised and non-ionised drugs confirmed the necessity of using biorelevant media in the development of new molecules such as the FaSSIF media. Besides the DCS was also confirmed as a suitability tool to help inform on the choice of formulation. In addition the design of experiment solubility study contributed to a better prediction of the expected *in vivo* behaviour regarding the categorisation of the studied drugs as dissolution-rate or solubility limited for intestinal absorption. Nevertheless given the

demanding effort of conducting a DoE in routine assessment a suggestion was made to use only the extreme DoE media conditions where the solubility shoud be at the minimum and maximum. No general pattern could be deduced since every drug displayed a unique profile. Additional studies would be necessary to revise and develop an even more sensible biorelevant DoE media and then drugs belonging to BCS class I and III should also be studied to determine if the *in vitro* pH-solubility profile of high soluble drugs is easier to predict.

The results of this study highlighted the difficulty to forecast the *in vivo* behaviour of BCS II drugs since the fasted intestinal milieu is constantly changing with variations of pH and surfactant concentrations. This complexity is augmented in the fed state with the composition of the meal content. As a conclusion solubility is clearly at the center of the drug development process as the high or low profile will first define the BCS classification. Then the BCS class will define the choice of formulation which will lead to the ability of the drug to be absorbed in the intestinal tract and eventually it will determine the pharmacokinetics and expected therapeutic effect. When developing a new drug the objective of the early stages is to anticipate whether the molecule is going to exhibit a consistent pharmacokinetic profile to achieve the pharmacological response with minimum inter-individual variability. Therefore one of the questions of this research project was to assess the direct impact of the *in vitro* biorelevant solubility on the *in vivo* pharmacokinetic behaviour. The variability observed in the *in vitro* solubility experiment could help forecasting the *in vivo* behaviour by informing on the pharmacokinetic variability using an *in vitro-in vivo* correlation (IVIVC) which will be discussed in the next chapter.

Chapter 4

Computational prediction of biorelevant solubility and influence on the *in vivo* plasma concentration profile

4. Computational prediction of biorelevant solubility of BCS class II drugs and influence on the *in vivo* plasma concentrations

4.1. Introduction

Over the last two decades there has been an increasing emphasis on the use of computational techniques to assist the development of drugs. This is due to the increasing knowledge in computer science and processing power and also the pressure to improve the efficiency of drug development, reduce *in vivo* studies (in both animals and man) and reduce the overall cost. Computer models are therefore extensively used to describe mechanistically the *in vitro* and physiological data in order to model the *in vivo* absorption, distribution, metabolism and excretion (ADME) of drugs. This approach is referred as physiologically based pharmacokinetic modelling (PBPK) which aims to link the *in vitro* tests results with the *in vivo* outcomes to establish an *in vitro in silico in vivo* correlation (*IVISIVC*) (Otsuka et al. 2013). Establishing an *IVISIVC* for BCS II and IV drugs is challenging since their very poor solubility characteristics are problematic for oral formulations. Indeed, aqueous solubility is a requirement for oral drugs although there is still a large number of molecules with poor water-solubility present within the drug discovery process (Hann 2011). When considering solubility and dissolution testing the use of biorelevant dissolution media (BDM) has now become the gold standard to establish *in vitro in vivo* correlation (*IVIVC*) and forecast the expected behaviour in human subjects. The simulated media must closely mimic the human gastrointestinal fluids as well as the solubilising capacities since it has been demonstrated that the gastrointestinal environment could improve the solubility of poorly soluble drugs and as a consequence their bioavailability (Sunesen, Vedelsdal, et al. 2005; Klein 2010). Various studies have shown that intrinsic aqueous solubility (S_0), *i.e.* solubility of the non-ionised species, can be well predicted (Norinder & Bergström 2006). The most notable one is the general solubility equation (GSE) first introduced in 1980 (Yalkowsky & Valvani 1980) and further revised by Ran and Yalkowsky (Ran & Yalkowsky 2001). This calculation is based on the lipophilicity value (log $P_{octanol:water}$) and the melting temperature (Tm). The former can be predicted computationally, and the latter is easily determined experimentally. However, the gastrointestinal tract is a dynamic environment where pH values vary from around 2 in the stomach to around 6.5 in the jejunum (Evans et al. 1988). Therefore, the tract environment has an important influence on the ionisation of pharmaceutical molecules as the solubility will change according to pH. This pH-dependent solubility can be calculated with the Henderson-Hasselbalch equation nevertheless it does not account for the solubilisation effect of the physiological amphiphile molecules (bile effect) present in the gut such as bile salt, phospholipids and monoglycerides. The introduction of the first fasted and fed state simulated intestinal fluids (FaSSIF and FeSSIF) in 1998 by Galia and coworkers are now commonly

accepted and include the bile salt sodium taurocholate and the phospholipid lecithin (Galia et al. 1998). Later, the addition of sodium oleate and monoglyceride in the second version of the fed state simulated intestinal fluid (FeSSIF-V2) (Jantratid et al. 2008) showed the importance of including this amphiphile and digestive products. The significant impact of sodium oleate has also been reported in a fasted state solubility study where it had a significant influence on all the investigated drugs (Khadra et al. 2015).

Biorelevant dissolution media have been extensively used in the early stages of development and in later stages for formulation studies however their potential as a support for computational predictions has not been fully investigated. Developing predictive computer models which consider pH-dependent solubility together with the bile effect requires a very good understanding of the complex mechanism of molecule solvation in the solvent associated with the ionisation properties of the amphiphiles (Bergström & Larsson 2018). As a result, it can significantly contribute to the discovery process and allow more informed decisions as whether to advance to the next stages in the development of new chemical entities. Only a few models have tried to predict the solubility of active pharmaceutical ingredients (APIs) in biorelevant media (Fagerberg et al. 2012; Bergström et al. 2010). These statistical models generally use the partial least square (PLS) regression technique also known as projection on latent structure which integrates features from the molecules to compute a principal component analysis (PCA) and multiple linear regression. A predictive model is also available in the commercial software ADMET predictor where biorelevant solubility in FaSSIF can be modelled (ADMET Predictor Simulation Plus, CA). Within Simcyp (Certara®) the Solubility In Vitro Analysis toolkit was developed (SIVA, Simcyp Ltd. Certara USA, Inc.). It is a parameter estimation tool which allows the estimation of intrinsic solubility, pKa and micelle partition coefficients by modelling aqueous and biorelevant solubility of drugs. In this study the Computational Oral Absorption Simulation tool developed in gPROMS (Advanced Process Modelling, PSE®) was applied and compared to the one implemented in Simcyp. The two models were used to predict the biorelevant solubility of the Orbito drugs since they include the effect of bile surfactants. The performance of the models were tested on their ability to predict the solubility in the fasted and fed state and the data were compared to the previously determined experimental solubilities in the full concentration range DoE study (chapter 2). Secondly a physiologically based pharmacokinetic method was utilised to predict the pharmacokinetic profiles of the drugs. The direct influence of the input solubility parameter in the PBPK model was assessed by predicting it with the FaSSIF and with different DoE values.

4.2. Materials and methods

4.2.1. Studied drugs

Nine drugs were included in this study and were taken from the previous design of experiment study (chapter 2) and the list was as follows; two acidic drugs (indomethacin and phenytoin), two basic drugs (zafirlukast and carvedilol), one ampholyte (aprepitant) and three neutral drugs (felodipine, fenofibrate and probucol). For practical reasons aprepitant was treated as a neutral compound since with its predicted pKa values of 2.8 (basic) and 9.7 (acidic) it is mostly non-ionised at the studied pH (5 and 7). The physicochemical properties of the drugs are listed in Table 15.

Table 15 Physicochemical properties of the studied drugs

Compound	**pKa**	**MW**	**Log P**	**S_o**	**Tm**	**PSA**
Indomethacin	4.5 (a)	357.7	4.27	0.0065	151	68.5
Phenytoin	8.3 (a)	252.2	2.47	0.010	286	58.2
Carvedilol [B]	7.8 (b)	406.4	4.19	0.010	114.5	75.7
Tadalafil	10 (b)	389.4	1.7	0.018	302	74.9
Zafirlukast [B]	4.3 (b)	575.6	5.4	0.0024	139	124
Aprepitant [B]	2.8 (b) / 9.7 (a)	534.4	4.5	0.0019	252	75.2
Felodipine	Not ionisable	384.2	3.8	0.011	145	64.6
Fenofibrate	Not ionisable	360.8	5.3	0.0047	80.5	52.6
Probucol	Not ionisable	516.8	10	2.7E-5	125	91.1

All data from Pubchem otherwise mentioned
[B] pKa values predicted with ACD/I-Lab (Advanced Chemistry Development, Inc.)
MW= Molecular weight in g/mol
LogP=Lipophilicity octanol/water
So=Intrinsic solubility (molar concentration) calculated with the GSE equation(Ran & Yalkowsky 2001)
Tm=Melting temperature in celsius degrees (°C)
PSA=Polar surface area in Ångström ($Å^2$)
(a) for acids and (b) for bases

4.2.2. In vitro experimental solubility measurements

The experimental solubility data measurements were taken from the previous determined data where an *in vitro* biorelevant media simulating the intestinal fasted and fed state was developed and a design of experiment approach was used to investigate the variability of solubility within this system (Perrier et al. 2018). The composition of the media is detailed in Table 16.

Table 16 Composition and concentration levels of the simulated biorelevant media (Perrier et al. 2018)

Parameter	Substance	Lower limit fasted (mM)	Upper limit fed (mM)
Bile salt	Sodium taurocholate	1.5	24
Lecithin	Phosphatidylcholine	0.2	4.8
Fatty acid	Sodium oleate	0.5	52
pH	Sodium hydroxide / hydrochloric acid	5	7
Salt	Sodium chloride	68	203
Buffer	Phosphate	15	45
Monoglyceride	Glyceryl mono-oleate	0.5	6.5

4.2.3. In silico models for solubility predictions

4.2.3.1. gCOAS version 1.3 (*model 1*)

In gCOAS the solubility ($S_{dissolv}$) in bile-micelle media at a specific gastrointestinal position is calculated by the modified Henderson-Hasselbalch equation (MHHE) (Sugano 2009). Solubility is considered at equilibrium and expressed by the following MHHE equations:

$$S_{dissolv} = S_0 \left(1 + \frac{[H+]}{K_a} + \frac{C_{bile}}{C_{water}} . K_{bm,0} + \frac{[H+]}{K_a} + \frac{C_{bile}}{C_{water}} . K_{bm,+}\right) \quad \text{Equation 25}$$

For monobasic drugs

$$S_{dissolv} = S_0 \left(1 + \frac{[H+]}{K_a} + \frac{C_{bile}}{C_{water}} . K_{bm,0} + \frac{[H+]}{K_a} + \frac{C_{bile}}{C_{water}} . K_{bm,-}\right) \quad \text{Equation 26}$$

For monoacid drugs

Where S_0 is the intrinsic solubility, $K_{bm,0}$ is the partition coefficient of the undissociated species, $K_{bm,+/-}$ is the bile micelle to water partition coefficient for mono-cationic and mono-anionic species respectively, C_{bile} is the concentration of bile acid, C_{water} is the concentration of water (55.56mM) and Ka is the dissociation constant). The value of $K_{bm,0}$ is defined according to the lipophilicity by the following relationship (Glomme et al. 2007; Avdeef et al. 1998):

$$\log K_{bm,0} = 0.74 \log P_{o:w} + 2.29 \qquad \text{Equation 27}$$

$$\log K_{bm,+} \approx \log K_{bm,0} - 1 \qquad \text{Equation 28}$$

$$\log K_{bm,-} \approx \log K_{bm,0} - 2 \qquad \text{Equation 29}$$

4.2.3.2. Simcyp In Vitro Analysis tool, SIVA version 2.0 (*model 2*)

The solubility model used in *SIVA* is described by equation 30 where S_0 is the intrinsic solubility, [BS] is the concentration of surfactant with a ratio of 1:4 between sodium taurocholate and lecithin, C_{H20} is the concentration of water (55.56mM), $K_{m:w,unionised\,/\,ionised}$ is the bile micelle to water partition coefficient and S_i is the aqueous phase solubility of the ionised form at a given pH. The equation combines a function for the aqueous phase solubility determined by the Henderson-Hasselbalch equation and a function for the bile micelle partition solubility of the drug. Total solubility $S_{(BS)Tot}$ is calculated by the combination of both the aqueous solubility (at a given pH) and the bile mediated solubility (at a fixed bile concentration).

$$S_{(BS)}\mathrm{Tot} = \left([BS].\frac{S_0}{C_{H2O}} . \mathrm{Km{:}\,w, unionised} + S_0 \right) + \left([BS].\frac{S_i}{C_{H2O}} . \mathrm{Km{:}\,w, ionised} + S_i \right)$$

Equation 30

Similarly to model 1 the estimation of the micelle partition coefficient for ionised species ($K_{m:w}$) is given by the equations 27, 28 and 29 developed by Glomme and coworkers.

4.2.4. Biorelevant solubility modelling

The dataset consisted of eight BCS class II drugs with diverse ionisation properties and a log P greater than 2 since an important solubilisation effect is expected for highly lipophilic drugs with poor solubility. Input physicochemical parameters were identical in each software as

listed in Table 15. The biorelevant media that the models were reproducing consisted of four different concentration levels of surfactant composed of bile salt (sodium taurocholate) and lecithin (lecithin). Two media represented the concentration levels in the fasted state (1.7 and 6.3 mM) and two represented the fed state (24.2 and 28.8 mM) according to the upper and lower concentration levels from Table 16. Biorelevant solubility was determined at pH 5 and 7 with the two modelling softwares and then the correlation factors (R^2) and the root mean square errors (RMSE) were calculated to evaluate the accuracy of the models by describing how close the predicted values were to the observed data as follows:

$$\mathrm{RMSE} = \frac{1}{\mathrm{N}}\sqrt{\sum_{\mathrm{i}=1}^{\mathrm{n}} (\mathrm{Pi} - \mathrm{Oi})^2} \qquad \text{Equation 31}$$

where *Pi* and *Oi* are the predicted and observed data points respectively and N is the number of data points.

4.2.5. PBPK modelling

PK-Sim version 7.2 (OSP Suite)

PK-Sim is a modelling software for the development of whole-body PBPK models. It provides useful tools to quantitatively predict the pharmacokinetic behaviour of drugs by describing the absorption, distribution, metabolism and elimination processes (ADME). The model integrates a large dataset of physiological and anatomical parameters in human (clinical) and animal (preclinical) species. The principle of PBPK modelling has been introduced in section 1.12 and the details of PK-Sim® with its methodology and structures have already been published elsewhere (Willmann et al. 2003).

4.2.5.1. Studied drugs

A PBPK model was built for five drugs, one acidic drug (indomethacin), two basic drugs (tadalafil and carvedilol) and two neutrals (felodipine and fenofibrate). For the remaining drugs the clinical pharmacokinetic data and/or drug physico-chemical and metabolism information were not sufficient or available in the literature therefore they could not be included. The physicochemical properties used for each drug are listed in Table 15 and the other input parameters such as metabolism, formulation are listed in Table 17 and Table 18.

Table 17 Other input parameters used for the PBPK models

Drug	f_{up}	Distribution model[c]	FaSSIF (mg/ml)	DoE Fasted Min - max (mg/ml)	Solubility gain per charge
Felodipine	0.01	(Poulin & Theil 2002)	0.053[d]	0.0008 - 0.14	na
Fenofibrate*	1%	na	0.0096[e]	0.00071 – 0.15	na
Fenofibric acid	*1%*	*(Rodgers & Rowland 2007)*	*1*	*na*	*1000*
Tadalafil	6%	(Berezhkovskiy 2010)	0.01	0.0039-0.015	100
Carvedilol	0.01	PK-Sim Standard[a]	0.136[d]	0.041 – 0.99	1.5
Indomethacin	0.01	(Poulin & Theil 2002)	0.435[e]	0.017 – 2.12	1E10

[c]The models developed by Poulin and Theil assume that the drug distributes homogenously into the tissue and plasma by passive diffusion accounting for nonspecific binding to lipids estimated from drug lipophilicity data and specific reversible binding to proteins present in plasma and tissue estimated from plasma protein binding. Rodgers and Rowland extended these equation models by incorporating ionization/charge considerations. These equations account for partitioning of unionized drug into neutral lipids and neutral phospholipids, dissolution of ionized and unionized drug in tissue water, electrostatic interactions between ionized drug and acidic phospholipids for strong ionized bases, and interactions with extracellular protein for neutrals, weak bases, and acids. The distribution model developed by Berezhkovskiy assumes a constant well-stirred perfusion limited model which leads to an equation for the organ tissue that differs from that previously published. The calculated pharmacokinetic profiles are particularly different for the organs with relatively large perfusion volume, and especially for drugs with small tissue-plasma partition coefficient and high blood-plasma concentration ratio.

[d](Söderlind et al. 2010)

[e](Clarysse et al. 2011)

[a](Willmann et al. 2005)

*Fenofibrate is a prodrug that is hydrolysed in the gut by a carboxylesterase (CES) into its active substance fenofibric acid.

FaSSIF; fasted simulated intestinal fluid at pH 6.5

DoE; design of experiment

F_{up}; fraction unbound in plasma

na; not applicable

4.2.5.2. Source of clinical PK data

A thorough search of the literature was conducted to collect available clinical pharmacokinetic studies in healthy volunteers and *in vivo* plasma profiles of the studied drugs (Table 18). When the individual data were not available but only the mean plasma profile was displayed it was scanned using the digitiser tool in the graphing software OriginPro® (OriginLab, Northampton, MA, USA).

Table 18 Formulations, metabolism and clinical data of the studied compounds

Compound	**Dosage (mg) / formulation**	**Healthy volunteers**	**metabolism**
Felodipine (Hardy et al. 1988)	10 / IR tablet	12 males	CYP1A2, 2C8-9-19, 2D6, 3A4
Fenofibrate** (Godfrey et al. 2011)	145 / IR tablet	54 (65% females)	CES2
*Fenofibric acid**	*na*	*na*	*UGT2B7, UGT1A3-6-9*
Tadalafil (Forgue et al. 2006)	20 / IR tablet	20 males	CYP3A4
Carvedilol (GlaxoSmithKline 2005)	50 / IR tablet	10 males	CYP1A2, 2C9, 2D6
Indomethacin (Alván et al. 1975)	100 / IR tablet	5 males	CYP2C9, 2B7

**Fenofibrate is a prodrug that is hydrolysed in the gut by a carboxylesterase (CES) into its active substance fenofibric acid.

4.2.5.3. PBPK Modelling strategy

The objective was to study the direct influence of the input intestinal solubility on the predicted *in vivo* plasma profile afer an oral administration of the drugs. Parametrisation of the models

started with the input of the drug's physicochemical properties (Table 15) along with other required parameters found in the literature such as metabolism pathways, dissolution profiles and the observed clinical study data (Table 17 and Table 18). Each model was first built using FaSSIF as input solubility parameter. When the model was validated with the FaSSIF data, input solubility was then changed to the minimum and maximum biorelevant values obtained with the DoE solubility study as listed in Table 17. In order to mimic the bile mediated variability of solubility observed in the biorelevant DoE study the "solubility gain per charge" module was adjusted accordingly (Table 17). The variability of PK observed in vivo was not available for all the drugs but the purpose was to investigate if the variability observed *in vitro* could help anticipate the variability of the *in vivo* plasma concentrations. Optimisation of the model using the parameter identification tool was employed on the metabolism input parameters and intestinal permeability values when needed.

4.2.5.4. Evaluation of the absolute performance of the PBPK models

Validation was first assessed by a visual check of the superimposed predicted versus observed data of the plasma concentrations profiles. In addition, a non-compartmental analysis was applied in Excel® using the PKSolver add-in program to determine the main PK parameters, area under the plasma concentration curve from zero to the last concentration point ($AUC_{0\text{-}tlast}$) (trapezoidal method), maximum concentration (C_{max}), time to maximum concentration (T_{max}) and half-life ($T_{1/2}$). The ratio of observed versus predicted values were calculated and presented as mean ratios$_{(Obs/Pred)}$ with a 95% confidence interval. A two-fold error range was set as a reference for an acceptable model prediction (Edginton et al. 2006; Parrott et al. 2011). Furthermore, three methods were used to analyse the predictive performance of the models as suggested by Sheiner and Beal and further revised by Wu (Sheiner & Beal 1981; Wu 1995). First a linear regression was performed for each drug and the correlation coefficient (R^2) was computed. Secondly, the percentage of prediction error (*pe %*) of each point was also calculated according to equation 32 and presented as a mean (*mpe %*) for each drug.

$$\text{pe } \% = \frac{\text{Predicted value} - \text{Observed value}}{\text{Observed value}} \text{ X } 100 \qquad \text{Equation 32}$$

Finally, the absolute performance was assessed by the evaluation of the precision of the predictive models. For each drug the calculated values of the percentage prediction error (*pe*

%) were divided into two groups: the positive (*+pe %)* and negative prediction errors (*-pe %*) and the results were presented as a mean for each bias (*+mpe % / -mpe %*).

4.3. Results

4.3.1. Biorelevant solubility modelling

Both models were able to predict the biorelevant solubility of all the drugs. The predictions were from observed values when solubility was purely predicted (RMSE>5, R^2 <0.6) therefore a fitting to the observed data was conducted. In SIVA an estimation of the bile-micelle partition coefficient from the observed solubilities was performed and in gCOAS the alpha and beta terms of equation 27 were estimated from the observed values. The goodness of fit plots for all the drugs are presented in Figure 25 where the overall prediction results are plotted (Figure 25a) along with the results by prandial state category (fasted or fed) (Figure 25b-c) and ionisation category (acid, base or neutral) (Figure 25d-f). The correlation factors (R^2) and calculated root square mean error (RMSE) values for the different categories and for the individual drugs are gathered in Table 19.

4.3.1.1. Results by drugs

The drugs individual RMSE values ranged from 0.062 to 1.35 for gCOAS and from 0.20 to 0.76 for SIVA (Table 19). The r-squared ranged from 0.14 to 0.95 for gCOAS and from 0.01 to 0.96 for SIVA. These results reflect the good performance of SIVA over its comparative model. Among all the data the solubility of phenytoin and zafirlukast were the best described by gCOAS (RMSE of 0.062 and 0.18 respectively) whilst SIVA performed the third best prediction for indomethacin (RMSE of 0.20). On the other hand, the weaker prediction was attributed to gCOAS for probucol (RMSE = 1.35). Similarly, the weaker prediction in SIVA was observed for the same drug (RMSE = 0.76) (Table 19).

4.3.1.2. Results by category

The RMSE values by category for gCOAS ranged from 0.20 to 0.86 while for SIVA the values ranged from 0.29 to 0.47 (Table 19). The r-squared values ranged from 0.41 to 0.95 for gCOAS and from 0.55 to 0.95 for SIVA. Overall the solubility prediction model implemented in SIVA performed better and it is evident on the goodness of fit plot where only two data points are falling outside the two-fold range limit. The model implemented in gCOAS demonstrated less consistency overall with more data points falling outside the limits, however it showed more accuracy to predict the biorelevant solubility in the fed state and for acidic drugs (0.28 and 0.20 respectively against 0.32 and 0.39).

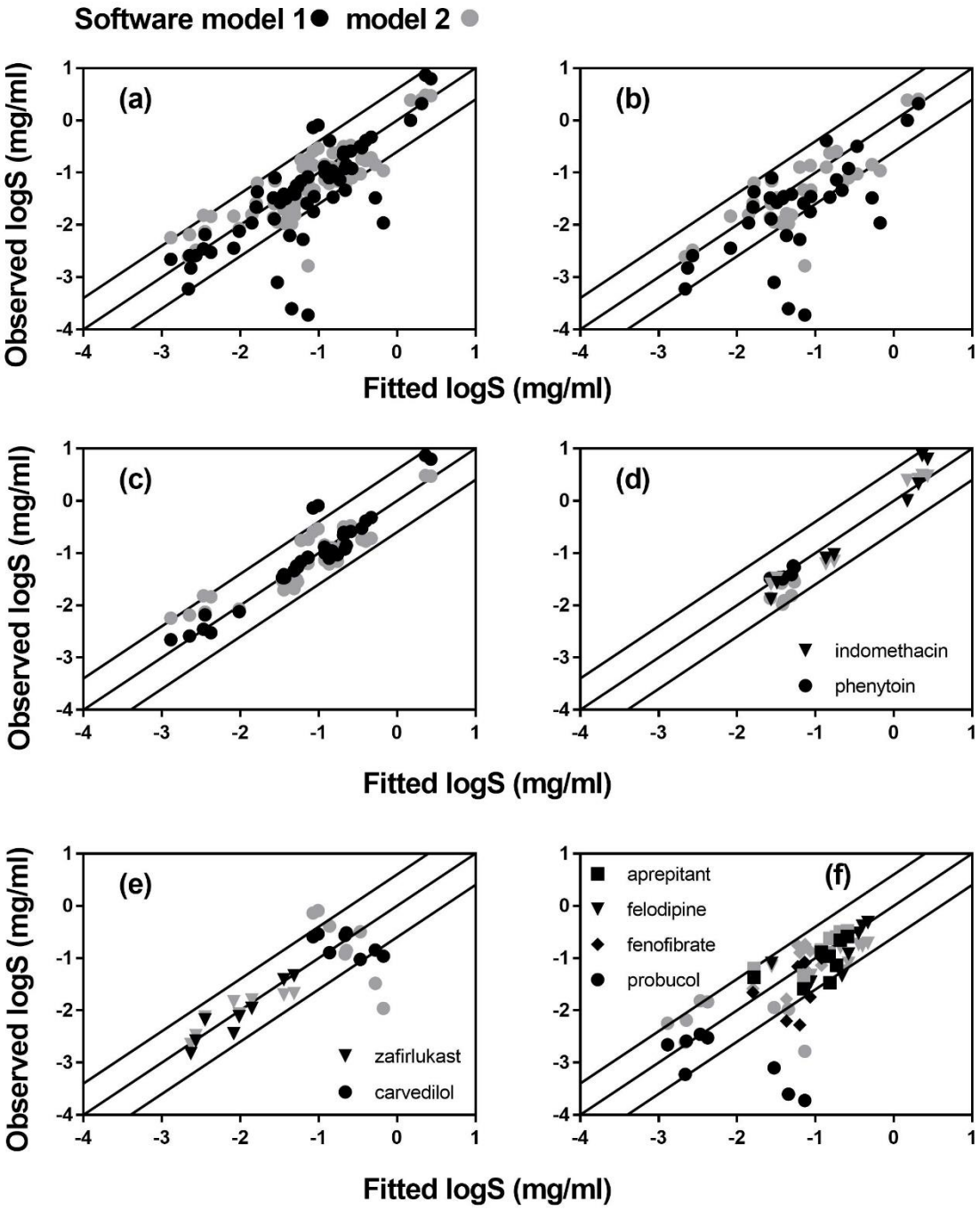

Figure 25 - Goodness of fit plots observed vs. fitted for the log solubility (mg/ml). (a) Overall, (b) Fasted state, (c) Fed state, (d) Acidic compound, (e) Basic compound and (f) Neutral compounds. Black symbols for results obtained with gCOAS (model 1) and grey symbols for SIVA (model 2). *Lines*, line of unity and two-fold error range.

Interestingly both models did not perform well in the fasted state as well as for the neutral drug category with the least calculated RMSE values of 0.86 - 0.77 for gCOAS and 0.47 – 0.47 for

SIVA respectively (Table 19). For both models the lowest RMSE values and therefore best predictions were obtained for the fed state and the acidic category.

Table 19 Calculated correlation factors (R^2) and calculated root square mean error (RMSE) values for the individual drugs and the different categories

	gCOAS (model 1)		**SIVA (model 2)**	
Drug	*R^2*	*RMSE (log unit)*	*R^2*	*RMSE (log unit)*
Indomethacin	0.95	0.28	0.96	0.20
Phenytoin	0.64	0.062	0.13	0.36
Carvedilol	0.82	0.91	0.39	0.46
Zafirlukast	0.88	0.18	0.80	0.21
Aprepitant	0.34	0.34	0.65	0.24
Felodipine	0.43	0.34	0.43	0.39
Fenofibrate	0.14	0.54	0.36	0.31
Probucol	0.63	1.35	0.01	0.76
Fasted	0.41	0.86	0.67	0.47
Fed	0.89	0.28	0.82	0.32
Acidic	0.95	0.20	0.95	0.29
Basic	0.48	0.66	0.79	0.36
Neutral	0.47	0.77	0.55	0.47
Overall	0.58	0.64	0.72	0.40

4.3.2. PBPK modelling

4.3.2.1. Simulation with FaSSIF solubility input

The comparison of mean simulated plasma concentration profiles versus mean observed profiles for the five drugs is shown in Figure 26a-e. The PBPK models managed to accurately simulate the plasma concentrations for all the drugs after an oral administration of an immediate release dosage form.

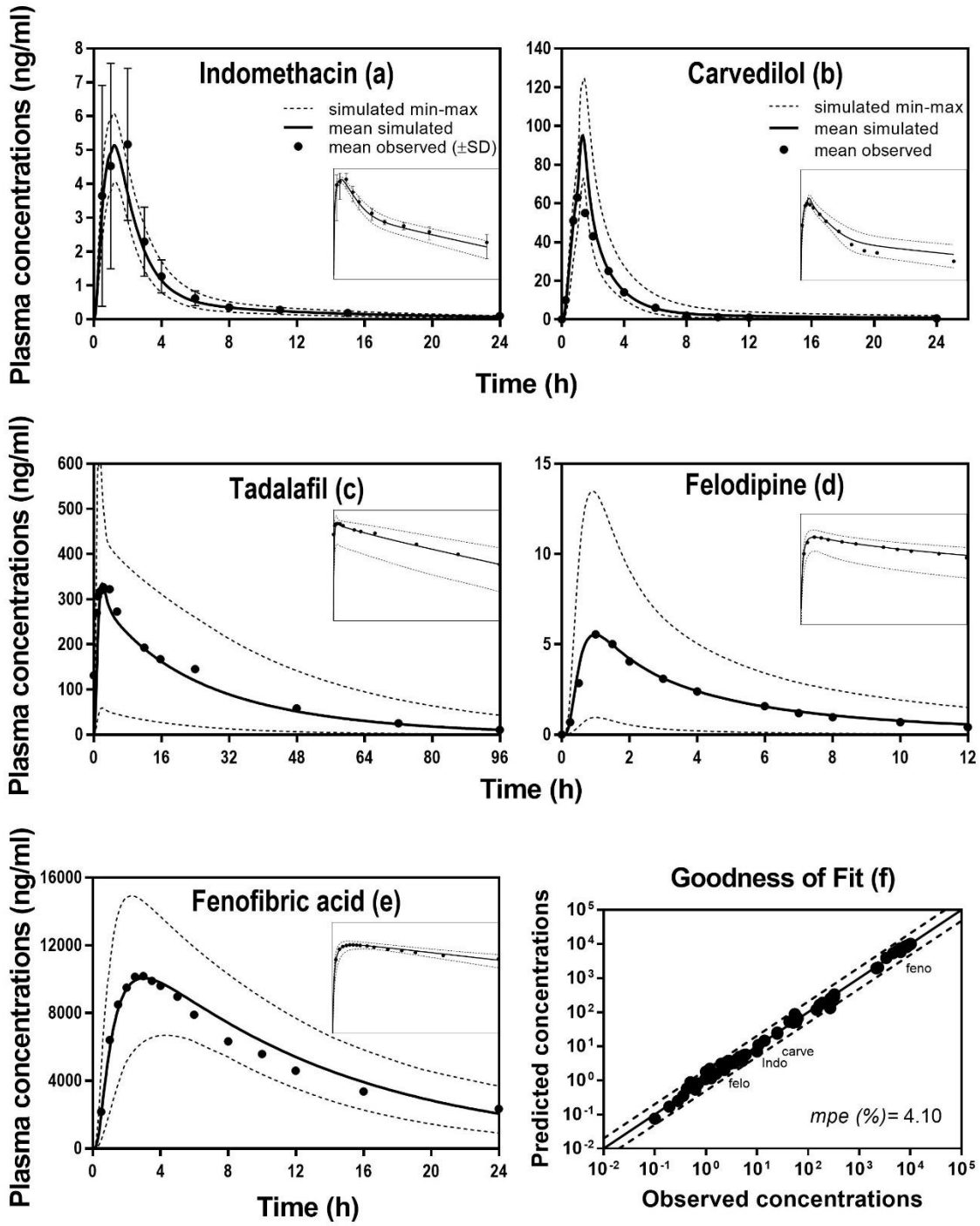

Figure 26 - **a – e** Comparison of simulated plasma profile concentrations (─ solid line; mean simulated, dashed lines; simulated minimum and maximum plasma profiles) and mean observed plasma profile concentrations (● dots; mean ±SD) after oral administration. The inserts display the same plot on a semilogarithmic scale. **f -** Goodness of fit plot for simulations of for all simulated vs. observed concentrations plot. *Line*, line of unity; *dashed lines*, twofold error range; *mpe (%)* mean percentage error.

The calculated mean ratio of observed over predicted of the PK parameters are presented in Figure 27 and they were all within the two-fold error range (20/20). The $AUC_{0\text{-}tlast}$ of felodipine was borderline (mean $AUC_{0\text{-}tlast}$ = 1.92) with a large variability (standard deviation = 2.24 and 95% Confidence Interval = 1.26).

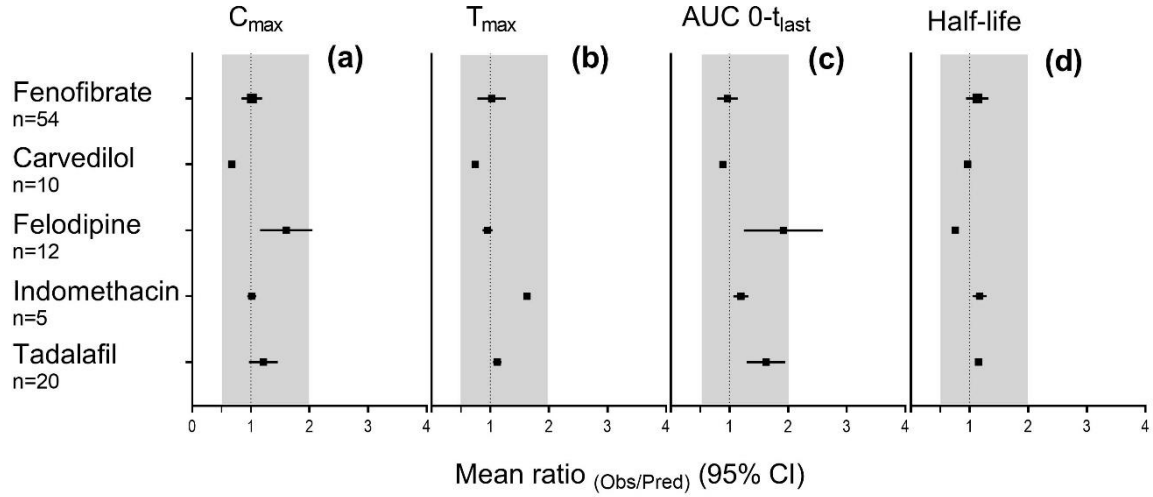

Figure 27 - Comparison between predicted and observed pharmacokinetic parameters. Results are reported as mean ratios (black squares) for each drug with a 95% confidence interval (horizontal lines). (a) Maximum concentration (C_{max}), (b) time to maximum concentration, (c) area under the plasma concentration-time curve (AUC 0-tlast) and (d) half-life

The goodness of fit plot (Figure 26f) and the calculated metrics indicated a good correlation factor (R^2) between predicted and observed concentrations for all the drugs with an overall r-squared value of 0.994 and a mean prediction error (mpe%) of +4.10 (Table 20). The individual R^2 values ranged from 0.868 for tadalafil to 0.982 for felodipine (Table 20). The individual mean prediction errors (mpe %) were also satisfactory for the whole set of drugs with calculated values below 12 % except for carvedilol where the mean value was 29.1%. Those results were homogeneous with the oriented bias expressed by the positive and negative prediction error (±*mpe %*) where all the values were below 14% (Table 20). The values were ranked between -13.9% for tadalafil and +12.8 for felodipine. Carvedilol showed a tendency to overpredict with a positive mean percentage error of +42.8% (Table 20). Interestingly, the neutral drugs felodipine and fenofibrate both yielded a similar profile with no important positive bias (+mpe % around 10) and very small negative prediction errors of -1.05 and -5.51 respectively.

Table 20 Predictive performance of the PBPK models with the calculated r-square and mean prediction errors

Drugs	R^2	Mean prediction error (mpe)		
		mpe %	+mpe %	-mpe %
Carvedilol	0.924	29.1	42.8	-11.9
Felodipine	0.982	9.34	12.8	-1.05
Fenofibrate	0.969	2.76	9.20	-5.51
Indomethacin	0.944	-11.6	8.37	-13.8
Tadalafil	0.868	-11.4	1.49	-13.9
Overall	0.994	4.10	20.4	-10.7

4.3.2.2. Simulation with variable solubility input

The graphic plots showing the direct influence of input solubility value on the simulated *in vivo* plasma concentration profiles are presented in Figure 28. Only tadalafil and felodipine are presented since changing the input solubility value for the other drugs did not influence the plasma concentrations. The boxplots expressing the variability of solubility in the DoE fasted biorelevant media are displayed and the single FaSSIF solubility value is highlighted (Figure 28a-b). The three resulting profiles from the input minimum and maximum DoE solubility (minimum and maximum whisker) and FaSSIF solubility values are displayed in Figure 28c-d. For tadalafil it is obvious that changing the input solubility had a significant influence on the *in vivo* exposure since the low plasma profile simulated was generated with the minimum DoE solubility value and the highest plasma profile with the maximum DoE solubility value (Figure 28c). The influence was less evident for felodipine where a significant decrease of the plasma exposure was observed when the minimum DoE solubility was used as input, but no variation was notable when input solubility was maximum. In fact, the simulated fraction absorbed (F) was already completed with the FaSSIF solubility value.

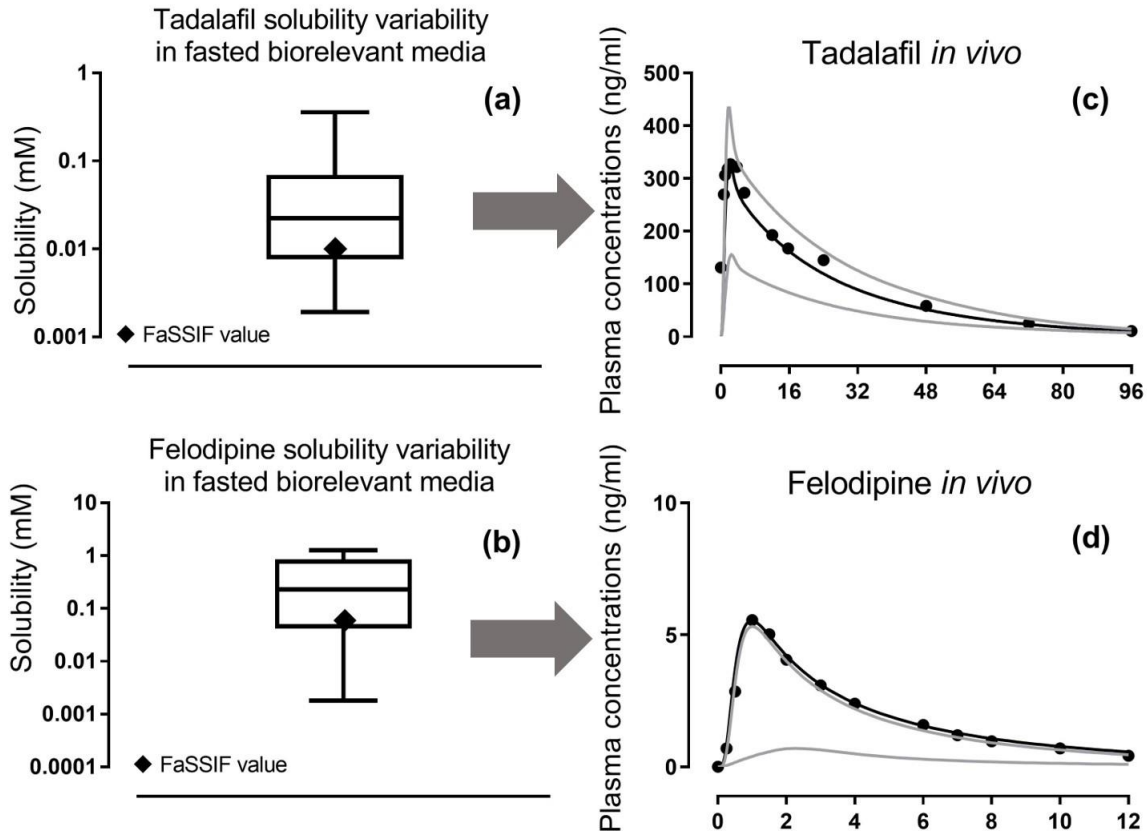

Figure 28 - Influence of the input solubility on the simulated plasma profiles. On the left-hand side variability of solubility is plotted as boxplot for (a) tadalafil and (b) felodipine. On the right-hand side the resulting plasma concentration profiles for (c) tadalafil and (d) felodipine (● dots; mean observed values, ─ black solid lines; mean simulated with FaSSIF solubility as input and ─ grey solid lines; simulated with min/max DoE solubility value as input).

The predicted pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\text{-}tlast}$ along with fraction absorbed (F) and bioavailability (BA) are given in Table 21. For tadalafil the calculated ratios of simulated with FaSSIF over simulated with minimum or maximum DoE value indicate the large influence observed. The pharmacokinetic parameters C_{max}, AUC, fraction absorbed, and bioavailability were halved with the minimum DoE solubility while with the maximum solubility they were augmented of around 30%. For felodipine the minimum DoE solubility significantly decreased the predicted exposure ($AUC_{0\text{-}tlast}$ divided by 6) because the fraction absorbed (F) and bioavailability were extensively decreased Table 21.

Table 21 Influence of the input solubility on the predicted pharmacokinetic parameters

	Tadalafil					
	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0\text{-}tlast}$ (ng/ml*h)	Half-life (h)	F	BA
FaSSIF	327.7	2.0	8185.2	20.0	0.68	0.47
Min DoE	155.8	2.5	4100.0	23.3	0.34	0.24
Ratio	*2.1*	*0.8*	*2.0*	*0.85*	*2.0*	*1.9*
Max DoE	436.1	1.8	10474.4	20.1	0.86	0.60
Ratio	*0.75*	*1.1*	*0.78*	*0.94*	*0.79*	*0.78*
	Felodipine					
	Cmax (ng/ml)	Tmax (h)	$AUC_{0\text{-}tlast}$ (ng/ml*h)	Half-life (h)	F	BA
FaSSIF	5.5	1.0	23.1	4.7	1.0	0.14
Min DoE	0.7	2.2	3.7	4.3	0.17	0.03
Ratio	*7.8*	*0.45*	*6.2*	*1.09*	*5.8*	*4.6*
Max DoE	5.3	1.0	23.1	4.3	1.0	0.14
Ratio	*1.0*	*1.0*	*1.0*	*1.0*	*1.0*	*1.0*

C_{max}; maximum concentration, T_{max}; time to maximum concentration, $AUC_{0\text{-}tlast}$; area under the curve, F; fraction absorbed, BA; bioavailability, FaSSIF; fasted simulated intestinal fluid

4.4. Discussion

4.4.1. Biorelevant solubility predictions

In this study two modelling software programs were investigated and evaluated on the computational prediction of intestinal solubility of a set of eight poorly soluble drugs. The predictions were compared to *in vitro* experimentally observed values from various biorelevant media systems. To achieve satisfactory predictions estimation of the bile-micelle coefficient had to be performed for SIVA (model 2) while for gCOAS (model 1) estimation of the equation terms alpha and beta had to be fitted to the observed values (equation 27). The results showed that for both models, predictions in the fed state were superior to the fasted state. Indeed, poorly soluble drugs generally exhibit a positive food effect due to the solubilisation

influence of higher amphiphile concentrations (Gu et al. 2007; Parrott et al. 2009) which seemed easier to forecast. In the fasted state the interplay between the intestinal fluid and the drug is more complex thus there is no obvious pattern and the solubility will depend more on the individual physicochemical properties of the molecules such as the lipophilicity and pKa. Interestingly, the models were able to accurately predict the solubility for the acidic group ($R^2 > 0.9$ and RMSE < 0.3), probably because for this group it is known that solubility in biorelevant systems is predominantly pH driven with very little impact related to biorelevant media components (Khadra et al. 2015; Zhou et al. 2017b; Perrier et al. 2018). Therefore, the Henderson-Hasselbalch parameter is dominant with little input from the remaining solubilisation parameters. According to the predictions achieved with our set of eight various drugs the weakness of the current gCOAS and SIVA models is predominant for the prediction of neutral compounds. Although the neutral group is not affected by ionisation properties the solubility enhancement relies essentially on the effect of the amphiphilic molecules which are subject to ionisation. Therefore forecasting the ionisation profiles and their influence of the different media additives such as bile salt, phospholipids remains the biggest challenge for prediction models. The complexity of the solubility mechanism could explain those results, which is highlighted in the findings of the published DoE studies (Khadra et al. 2015; Zhou et al. 2017b) where for basic and neutral drugs, pH and the multiple amphiphilic media components (sodium oleate, bile salt and phospholipid) have an equivalent standardised effect on solubility. In general, the non-ionised BCS II drugs are the most problematic since they exhibit the lowest intrinsic solubility but on the other hand they benefit from the biggest solubilisation effect in the intestinal fluid. Results of the prediction for this group (Figure 25f) show a tendency to underestimate this effect by underpredicting the biorelevant solubility. However, it should be noted that the biorelevant media used for the experimental solubility included fatty acid (sodium oleate) and monoglyceride (glycerol-mono-oleate) while the models only account for bile concentration which is represented by bile salt and lecithin. In addition, the influence of sodium oleate on the intestinal solubility has been previously demonstrated for BCS class II drugs therefore it could be a valuable parameter to consider including in the *in silico* tools.

Based on the results obtained in this study a linear regression equation could be made by drug category and prandial state to calculate better estimate of the alpha and beta terms of Equation 27. This would be more sensible considering the additional amphiphile sodium oleate and the digestive product monoglyceride on top of bile salt and phospholipid represented in the developed biorelevant media. The estimated terms could be tested on further solubility predictions in the fed state and for acidic drug since they exhibited satisfactory results.

4.4.2. PBPK modelling of the plasma concentration profiles

For drugs administered orally aqueous solubility and dissolution are essential parameters. Indeed, the administered formulation must dissolve in the gastrointestinal tract then the active pharmaceutical ingredient has to be solubilised before permeating through the intestinal epithelium (Sim 2015). In order to simulate these processes mechanistic models have been developed and implemented into PBPK software (Kostewicz et al. 2014; Jones & Rowland-Yeo 2013) an approach that has been rapidly developing over the last three decades and has demonstrated successful results permitting better informed decisions regarding clinical development and regulatory communications (Jones et al. 2009; Jones et al. 2015; Rowland et al. 2011). When associated with *in vitro* biorelevant dissolution results the ADME of drugs can be simulated quantitatively. This approach is beneficial for poorly soluble drugs (BCS II) since the factor limiting absorption is often the dissolution in the small intestine. In fact most of the reported PBPK models concern poorly soluble and highly permeable drugs for instance nifedipine (Wagner et al. 2013), fenofibrate (Juenemann et al. 2011), troglitazone (Nicolaides et al. 2001) and etoricoxib (Okumu et al. 2009). For this class of drugs, the use of biorelevant media that reproduces the solubilising effects of the bile secretions can enhance solubility and dissolution and hence absorption (Klein 2010). In addition the effect of food and the fat content of the meal can also contribute to improve the solubility and dissolution of poorly soluble drugs (Persson et al. 2005). However, it is not always the case and for some drugs absorption can be reduced in the presence of food, for example ciprofloxacin, atenolol or verapamil (Singh 1999; Fleisher et al. 1999). While many studies focus on the correlation of *in vitro* dissolution with the *in vivo* pharmacokinetic, only a few studies have investigated the direct influence of poor solubility on the plasma concentration profile. Yet poorly soluble drugs are more likely to exhibit large inter and intra-individual variability compared to BCS I and III (Daublain et al. 2017).

In this study the successful simulation of all the plasma concentrations profiles using PBPK modelling confirms the ability of this approach to be a useful tool in the drug development process (Sager et al. 2015). Results showed that for tadalafil and felodipine (2 out of 5 drugs) variable input obtained from biorelevant solubility had an important influence on the *in vivo* plasma concentrations. Many studies have demonstrated the impact of the biorelevant media composition on the predicted plasma profile of poorly soluble drugs (Nicolaides et al. 2001; Wagner et al. 2012) but the impact of the variability in solubility has not been fully investigated. Changing the input solubility value for indomethacin, carvedilol and fenofibrate did not influence the plasma concentrations. In fact, for these drugs the predicted fraction absorbed in the intestinal tract was maximal (Fa = 1) even with the minimum solubility input values. Despite their poor water-solubility those compounds are readily absorbed in the intestinal tract

because of the compensatory effect of high permeability along with their high lipophilicity. Nevertheless, the drug carvedilol is subject *in vivo* to an extensive first-pass metabolism while indomethacin and fenofibrate exhibit *in vivo* an important inter and intra-individual variability (Helleberg, 1981; Miller & Spence, 1998; Morgan, 1994). Although the dataset of drugs investigated had various ionisation properties it may be necessary to evaluate thses findings on a larger test set of drugs. In addition it is of interest to make a step further in the use of biorelevant media to be able to anticipate the *in vivo* solubility not only in an average subject (*e.g.* single FaSSIF or FeSSIF) but also in a population of individuals. It might not be fully applicable in the early stages of development as a standard screening method but at least the extreme media conditions (minimum-maximum) could be included. *In vitro-in vivo* extrapolation (IVIVE) could possibly be made by linking the variability of solubility *in vitro* and the *in vivo* variability observed between subjects. This would be particularly beneficial for BCS II and IV since they demonstrate large *in vivo* variability.

4.5. Conclusion

The computational models studied herein performed reasonably well at predicting the biorelevant solubility of poorly soluble drugs as well as simulating their corresponding *in vivo* plasma profiles. The importance of using appropriate biorelevant dissolution media in the drug discovery process and development has been highlighted once more. These media have been extensively used and acknowledged as the gold standard for *in vitro* solubility and dissolution studies in the industry to demonstrate *in vitro in vivo* correlations (IVIVC) however their potential usefulness for computational predictions still needs to be fully investigated, especially for BCS II and IV molecules. In this study two computer models were compared on their ability to forecast the biorelevant solubility of poorly soluble drugs. The SIVA model implemented in Simcyp performed better overall but on the other hand the gCOAS model showed more accuracy. Interestingly both models produced satisfactory fitting results for the acidic drug and in the fed state however the prediction of the neutral drug and in the fasted state showed some limitations. This outcome highlighted the need for improvement of *in silico* models for solubility prediction in particularly with the development of more sophisticated models for the integration of the multiple components present in the human intestinal fluid. Indeed the pH effect for acidic drug seems well understood as well as the food effect for poorly soluble drugs. However the interplay between the drugs and the intestinal components in the fasted state still need to be investigated.

Regarding the PBPK modelling PK-Sim® demonstrated good results for the prediction of plasma concentration profiles of the studied drugs. On the other hand the direct influence of the input solubility parameter was assessed. The objective was to test whether the observed

variability in biorelevant solubility could be predicted with an IVIVE approach. Interestingly for two drugs (tadalafil and felodipine) changing the input solubility produced a significant variation of the predicted PK profile but it could not be correlated with the *in vitro* solubility. Therefore a suggestion is made on the use of biorelevant dissolution media where the integration of multiple biorelevant levels instead of only one single FaSSIF could be of interest to help anticipate the *in vivo* variability of drug exposure between individuals.

Chapter 5

Physiologically based pharmacokinetic modelling of a phase I clinical trial of a cancer drug

5. Physiologically based pharmacokinetic modelling of a phase I clinical trial of a cancer drug

5.1. Introduction

During the development of anticancer drugs phase I clinical trials are crucial since they represent the first administration of a new drug to humans. The purpose of phase I clinical trials is to determine the appropriate dose and/or schedule of dosing of the drug to be able to go into the phase II trials where the efficacy of the drug will be tested. As stated in the S9 ICH guidelines (ICH Expert Working 2009), the start dose for first administration in humans of systemically administered small molecules is generally extrapolated from interspecies scaling of the animal doses. The further successful clinical drug development will depend on the results hence the design of the trials must be comprehensively and cautiously organised. Typically, phase I clinical trials in oncology require a small cohort of patients. The design is single-armed, open labelled and sequential meaning that there is only one group of patients, both the researcher and the patient are aware of the treatment being given and the treatment is administered by escalating dose. When designing such trials the researchers must consider many aspects including the starting dose, the dose increment, the dose escalation method, the number of patients per dose level, the definition of the maximum tolerated dose (MTD), the dose-limiting toxicity and target toxicity level and finally the patient selection. Patients included in the trials are patients whose cancer is progressing despite the standard treaments but they still show good performance status. Although the trials do not require many patients the recruitment can take a long time to be completed.

The fundamental principle in dose escalation of cancer drugs is to avoid exposure of the patient to unnecessary subtherapeutic doses meaning that as many patients as possible must receive a therapeutic dose while maintaining safety and rapid dose increases. There are two types of designs for phase I clinical trials in the development of cancer drugs. The rule-based design and the model-based design. In rule-based designs the researchers use observations of target events (*e.g.* dose-limiting toxicity) from the clinical data of the patients to assign *a prori* each patient to a dose level. The appropriate dose for the phase II trials is also determined *a priori* by the rules. In the model-based designs a model describing the dose-toxicity relationship is used to estimate the target toxicity and assign patients to a dose level. However, there is a higher risk of overexposure of patients with the model-based designs therefore a set of specific restrictions has been established to limit the exposure of patients to harmful doses.

These types of design methods were developed when both efficacy and toxicity of cytotoxic drugs were assumed to increase with the dose. As a consequence toxicity was the reference for the maximum limit of exposure. Then with the introduction of targeted cancer therapies it

was demonstrated that toxicity and efficacy were not systematically connected and efficacy could be observed at doses where significant clinical toxicity were not observed (Korn et al. 2001; Cannistra 2008). For this new class of drugs toxicity as a reference was replaced by drug related-biological effects.

5.1.1. Rule based designs

The specificity of the rule-based design is that it does not rely on a dose-toxicity relationship assumption. The different rule-based designs are characterised by the "up-and-down" method as they allow escalation of the dose as well as a de-escalation (Le Tourneau et al. 2009) (Figure 29-A). The principle of the method is to increase or decrease the dose depending on the reactions in the previous cohort of patients (Figure 29-A). If no severe toxicity is observed in the treated cohort then the dose is increased however if a severe toxicity is observed the dose is reduced by a fraction of the preceding dose (Le Tourneau et al. 2009). This method leads to a final dose which represents a probability of around 50% of severe toxicity. The most used rule-based design is the traditional 3+3 design (Storer 1989) (Figure 29-B). This design is conducted with cohorts of three patients. The first cohort receives the starting dose and the following cohorts receive predefined increased dose levels. In a cohort of patients if no dose-limiting toxicity is observed among the three patients, another group of three patients will receive the next dose. However, when at least one patient experiences dose-limiting toxicity, three new patients will receive the same dose level. The study is continued and the appropriate dose is the one where at least two patients among a cohort of three to six experience dose-limiting toxicities (*i.e.* 33% of patients).

Theoretically the dose escalation is calculated according to the Fibonacci sequence which means that the increment decreases as the dose increases (the second dose increases by 100% of the starting dose and then by 67%, 50%, 40% and 30-35% of the previous dose).

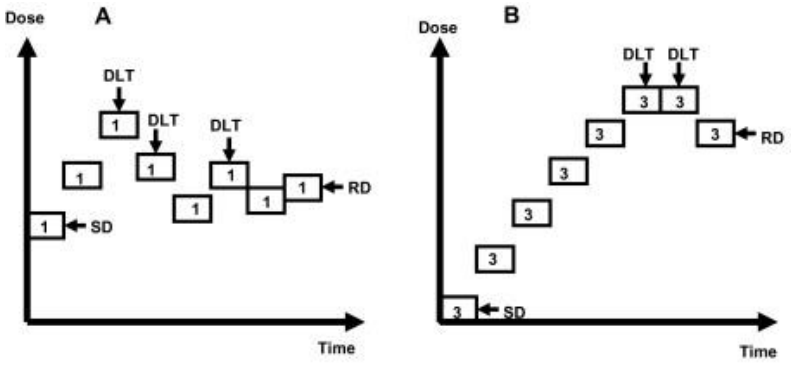

Figure 29 - Description of dose escalation designs for a phase I clinical trial in cancer drug development. The boxes represent a cohort with the number of patients receiving a dose level.

A) represents the general “up-and-down” method and B) represents the traditional 3+3 method. SD = starting dose, DLT = dose-limiting toxicities and RD = recommended dose or appropriate dose for phase II trials (Le Tourneau et al. 2009).

5.1.2. Model-based designs

Model based designs use statistical models to calculate a dose level that exhibits a specific probability of dose-limiting toxicity (Le Tourneau et al. 2009). The statistical models integrate toxicity data from the selected patients to produce a very specific dose-toxicity curve. Bayesian distribution models are generally applied. Bayesian models require an initial estimate of the distribution (prior distribution) which defines the shape of the toxicity curve. Then the occurrence or absence of toxicity observed in patients at each dose level helps informing the statistical model to adjust the initial estimate to a posterior distribution. The posterior distribution is evaluated to identify the dose closest to the toxicity level which will be used for future patients and also to set the recommended dose in phase II trials. (Le Tourneau et al. 2009).

5.1.3. PBPK modelling in an oncology population

The use of physiologically based pharmacokinetics has grown significantly in drug development for the prediction of pharmacokinetics in humans. PBPK models integrate all the properties of the human and animal physiology in a mechanistic system along with drug-related properties such as the physicochemical properties, absorption and metabolism properties (section 1.12). PBPK allows a quantitative modelling and simulation of the ADME processes of drugs in virtual populations and the study of variations on the drug exposure. The studied variations can be related to the population such as the age, the genetics or the organ functions or they can be unrelated to the population such as the effect of food or the interaction with other drugs. This approach has been extensively used at the beginning of PBPK modelling for the prediction of variations such as the study of drug-drug interactions, food effect, formulation effect and over the years an increasing focus has been made on the study of the impact on specific populations. Many publications have been predicting pharmacokinetics in the paediatric population by scaling down from the adult population, the effect of organ impairment, genetic variations or ethnicity on pharmacokinetics. In light of this growing interest the regulatory authorities have been acknowledging and accepting this

approach as part of the regulatory submissions, for example both the EMA[9] and FDA[10] are addressing the use of modelling and simulation in their updated DDI guidance.

The pharmacokinetics of drugs can be affected by several characteristics of the population. For instance organ volumes can be affected by the sex, body weight and body surface area which will impact the enzyme abundance and thus the metabolism of a drug. The plasma proteins and hematocrit levels also vary with the age of the subject which will affect the distribution and clearance (Jamei et al. 2009; Woo et al. 1994). In the cancer population it is generally acknowledged that the following physiologic parameters are affected compared to a healthy population; age, body weight and plasma protein levels (Veering et al. 1990; Alibhai & Horgan 2011; Engeland et al. 2005). Recently the PBPK software Simcyp (Certara®) has introduced a virtual oncology population to simulate the PK of drugs in this specific population (Cheeti et al. 2013). On the other hand for other PBPK software, when a specific population is not available it is possible to change the parameters of the healthy volunteer population according to the desired characteristic of a population. This is the case for PK-Sim® which was used in this study to build a whole-body PBPK model for a cancer drug. The PK data of the phase I dose escalation study were available and the objective was to develop a model to closely reproduce the clinical study in cancer patients and predict the dose escalation.

5.2. Materials and methods

5.2.1. The molecule and drug substance

The molecule AT13148 is the dihydrochloride salt of the S-enantiomer form of another molecule (AT9821) and is depicted in Figure 30. It is an AKT/Protein kinase B inhibitor that also blocks the activity of other selected AGC kinases (Saxty et al. 2007). The drug substance of AT13148 was kindly provided by Cancer Research UK (CRUK) along with the physico-chemical properties and in vitro data. They are listed in Table 22.

[9] EMA, Committee for Human Medicinal Products (CHMP). Guideline on the investigation of drug interactions [Final]. European Medicines Agency: London; 2012.

[10] FDA, Center for Drug Evaluation and Research (CDER). Guidance for industry: drug interaction studies-study design, data analysis, implications for dosing, and labeling recommendations [Draft Guidance]. U.S. Department of Health and Human Services, Food and Drug Administration: Rockville, MD; 2012.

Table 22 Physicochemical properties and in vitro data of AT13148 (CRUK reports)

parameter	value
MW (g/mol)	386.7
pKa	1.64(a) / 8.57(b) / 11.79(a)
Human plasma protein binding (%)	97.3
Apparent permeability (Caco-2 A-B / B-A) (cm/s)	0.48 / 3.58 x10^{-6}
Human liver intrinsic clearance (mL/min/kg)**	0.175

All data provided by CRUK

MW = molecular weight

(a) for acid and (b) for base

A-B / B-A = Apical to basolateral and basolateral to apical

**From *in vitro* human liver fractions in post mitochondrial (S9). In vitro intrinsic clearance was calculated with the following equation:

$$\text{Intrinsic clearance } (CL_{int}) \text{ (µl/min/mg protein)} = \frac{V \times 0.693}{T1/2} \qquad \text{Equation 33}$$

where V is the volume of incubation (µL) divided by the amount of protein in incubation (mg) and the half-life ($T_{1/2}$) is calculated from the elimination rate constant of the disappearance of the drug in the S9 fraction as a function of time. In vitro clearance was then scaled up to human liver intrinsic clearance with hepatocellularity and liver weight.

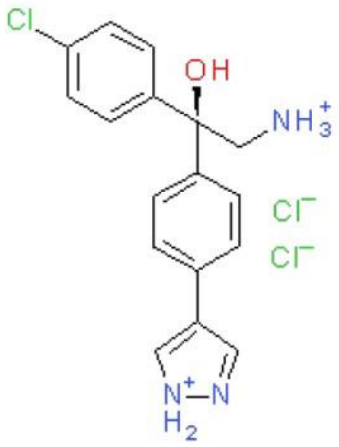

Figure 30 - Chemical structure of AT13148

5.2.2. Biorelevant solubility measurements

Upon reception of the drug substance the biorelevant solubility was measured experimentally in FaSSIF. A volume of 0.5 litre of fresh media was prepared according to the protocol from biorelevant.com. The phosphate buffer was prepared by adding 0.210g of sodium hydroxide, 1.977g of monobasic sodium phosphate monohydrate and 3.093g of sodium chloride into a 0.5 litre volumetric flask and volume was made up with deionised water. Then pH was adjusted to 6.5 and a weight of 1.120g of FaSSIF powder was added to the media. The volumetric flask was shaken and pH adjusted again. A volume of 4 ml was added to a 15 ml corning tube with a visual excess amount (10 mg) of solid drug AT13148. The tube was placed on a tube rotator at 40 rpm for incubation at 37°C for 24 hours. After 24 hours, a volume of 1 ml was transferred to an Eppendorf® and centrifugated at 15 000 rpm for 5 minutes. Following centrifugation 0.5 ml of the supernantant was transferred to an HPLC vial and solubility was analysed using the same HPLC-UV method detailed in section 2.2.3.4. The FaSSIF solubility was determined in triplicate.

The biorelevant solubility of the drug was also measured experimentally using the DoE method as described in chapter 2. The composition of the biorelevant media is presented in Table 23. The equilibrium solubility of AT13148 in these media was determined and the factors influencing its solubility were calculated statistically using Minitab®.

Table 23 Composition and concentration levels used in the DoE solubility measurements

parameter	**substance**	**Lower limit fasted (mM)**	**Upper limit fed (mM)**
Bile salt	sodium taurocholate	1.5	24
Lecithin	phosphatidylcholine	0.2	4.8
Fatty acid	sodium oleate	0.5	52
pH	sodium hydroxide/hydrochloric acid	5	7
Salt	sodium chloride	68	203
Buffer	Phosphate (KH_2PO_4)	15	45
Monoglyceride	glycerol mono-oleate	0.5	6.5

5.2.3. The studied population

The cancer population of the clinical study consisted of 56 patients with 55% female. The average age in years and weight in kilograms were 59.7 and 72.7 respectively. The studied population was spread in 9 cohorts of patients with 3 to 7 patients in each cohort.

5.2.4. Clinical PK study

The PK data were taken from the phase I clinical trial of the AKT/Protein kinase B inhibitor AT13148, given orally in patients with advanced solid tumors. The study consisted of a dose escalation to evaluate the safety, efficacy and recommended dose of AT13148. Each cohort of patients received a single dose with a starting dose of 5 mg and the subsequent doses were as follows: 10, 20, 40, 80, 160, 180, 240 and 300mg.

5.2.5. Modelling development and evaluation

The aim of this work was to build a PBPK model to simulate a phase I clinical trial study of a cancer drug in cancer patients. The PBPK model of AT13148 was built using PK-Sim® version 7.2. In order to create a virtual cancer population a customisation of a healthy population was carried out. The haematocrit and albumin levels were changed according to the publication of Cheeti and colleagues (Cheeti et al. 2013). In PK-Sim® the healthy population can be exported to a CSV format and opened in Excel®. The Excel® file contains all the anatomical and physiological parameters of each individual which can be amended. Therefore each cohort was created according to number of patients and proportion of female, the CSV files were exported, modified according to the individuals age, height, weight, body mass index (BMI) and re-imported in the software. The haematocrit value was set to 35.8% for females and 37.7% for males and the albumin levels were calculated for each individual according to equation 34 (Cheeti et al. 2013):

$$z = y_0 + ax + by \qquad \text{Equation 34}$$

where z is albumin, x is age, y is BMI, y_0 is intercept; a and b are the parameters of the slope. All input parameters and data used in the development of the model are listed in Table 24. The model was built using FaSSIF solubility as input and the solubility gain per charge was adjusted to reflect the pH-dependent solubility observed with the DoE experiment. The transcellular permeability was set to $7.6.10^{-5}$ cm/s and the intestinal permeability was calculated by the model from the lipophilicity and effective molecular weight properties. PK-Sim assumes a constant permeability value along the intestinal tract but to allow a pH-effect

on the permeability the pH- and pKa- dependent penalty factor was selected. The default distribution model (PK-Sim standard) was preferred as it performed better than the other distribution models. The transporter protein ABCB1 (P-gp) was added to the model since the Caco-2 experiment suggested an efflux process in the intestinal tract. Parameter estimation was utilised to optimise the input values of the transporter as no quantification studies were available. The permeability value and liver intrinsic clearance were also optimised separately with the parameter estimation tool to improve the fit to the observed data. The Levenberg – Marquardt algorithm was used for the parameter estimation. The estimation of the transporter kinetic V_{max} and K_m were performed simultaneously with a total of 15 evaluations and a total error of 1.24. For the permeability the model ran 15 evaluations and returned a total error of 0.24 while for the plasma clearance 13 evaluations were done with a total error of 0.79.

The objective was to validate the model at a 10 mg dose and then scale down the dose to 5 mg and up to 300mg. The model was first evaluated against the mean observed PK profile at 10 mg of the clinical study. A non-compartmental analysis was applied in Excel® using the PKSolver add-in program (Zhang et al. 2010) to determine the main PK parameters, area under the plasma concentration curve from zero to the last concentration point ($AUC_{0\text{-}tlast}$) (trapezoidal method), maximum concentration (C_{max}), time to maximum concentration (T_{max}) and half-life ($T_{1/2}$). The mean predicted PK parameters (C_{max}, T_{max}, $AUC_{0\text{-}tlast}$ and half-life) were compared to the respective mean observed PK parameters and 20% difference between predicted and observed was used as the limit criteria.

Single simulations were first performed with an average individual to fit to the mean observed PK profile and conduct the optimisations. Once the single simulation was acceptable (within 20% difference of the PK parameters) the population simulation was performed with the corresponding number of patients in the cohort.

Table 24 Input parameters and data for the PBPK model of AT13148

Parameter	Input value	Source

Physicochemical properties		
Molecular weight	386.7 g/mol	CRUK
Log $P_{o:w}$	2.16	Predicted using ACD/lab
pKa1 (acid)	1.64	CRUK
pKa2 (base)	8.57	CRUK
pKa3 (acid)	11.79	CRUK
Fraction unbound in plasma	2.7 %	CRUK
Permeability		
Transcellular permeability	7.4 10^{-6} cm/s	Estimated
Solubility		
FaSSIF (pH6.5)	0.00232 mg/ml	Experimentally determined
Formulation		
IR Oral caspules**	Dissolved	CRUK clinical study
Transport		
ABCB1 (Km / Vmax)	0.68 µM / 0.14 µM/min	Estimated
Distribution		
PK-Sim standard method	PK-Sim standard method	(Willmann et al. 2005)
Elimination		
Liver plasma intrinsic clearance	0.0175 ml/min/kg	CRUK

MW = molecular weight

logPo:w = lipophilicity octanol/water

FaSSIF = fasted simulated intestinal fluid

IR = immediate release

**Because of its very poor water solubility no solid formulation was appropriate therefore the drug was dissolved in polyethylene glycol (PEG) and the solution filled into capsules.

5.3. Results and discussion

5.3.1. Biorelevant solubility measurements

According to its solubility experimentally determined and the calculated permeability AT13148 could be assigned to the BCS class IV. It is an ionisable drug with three pKa values and therefore it will have a pH-dependent solubility. The predicted lipophilicity of AT13148 (logP = 2.16) is moderate and considered favourable for oral administration. At physiological pH values the drug is basic therefore a good solubility in an acidic environment (*e.g.* gastric fluid) is expected. However due to its limited permeability the bioavailability is expected to be low *in vivo* with a dose dependent behaviour. The biorelevant solubilities of AT13148 in FaSSIF and DoE media are plotted in Figure 31. The mean solubility determined in FaSSIF media was 0.00232 mg/ml. This value is very low given the fact that biorelevant media components such as bile salt and lecithin found in FaSSIF media were expected to have a large positive effect on a poorly soluble drug. Nevertheless the moderate lipophilicity of AT13148 (logP = 2.16) indicated that a moderate solubilisation effect from biorelevant media components could be expected. Indeed drugs with lipophilicity greater than 2 (logP > 2) are known for showing an important solubilisation effect because the presence of surfactants lower the surface tension and help increase the dissolution. (Savjani et al. 2012). The mean solubility results of the DoE study in the fasted and fed states at pH 5 and 7 were greater than the FaSSIF solubility results which show that a greater solubilisation effect took place in this biorelevant media. This outcome is associated with BCS IV drugs which generally profit from a significant positive food effect but with a variable bioavailability (Ghadi & Dand 2017). In addition the mean DoE solubility was higher at pH 7 compared to pH 5 Figure 31. According to its pKa values, the drug is mostly ionised between pH 5 and 7 therefore this should facilitate its dissolution. However the complexity of the influence of the simulated intestinal fluid on poorly soluble drugs has already been reported (Khadra et al. 2015; Zhou et al. 2017b) thus the higher solubility observed at pH 7 could be explained by the partitioning of a fraction of non-ionised drug into micelles formations near the basic pKa value of 8.57.

The statistical standardised effect of the individual factors and factor interactions on the equilibrium solubility of AT13148 are presented in Figure 31b. Among the individual factors bile salt was the only significant outcome. For the factor interactions a significant effect was found between lecithin and buffer and between soldium oleate and pH. Surprisingly no individual factor such as sodium oleate or pH had a significant effect whilst the presence of bile salt significantly reduced the solubility of the drug Figure 31b. For ionisable drugs pH-dependent solubility is generally the most important factor especially for acidic and basic compounds. On the other hand the pKa values of AT13148 were outside of the pH range of the DoE study and as a consequence the drug was mostly ionised. Interestingly the negative

effect of bile salt on solubility had already been demonstrated for the basic drug carvedilol (Khadra et al. 2015) and furthermore a delayed solubility and a reduced pH dependency of an ionisable drug was reported with an anionic surfactant such as sodium taurocholate (Chakraborty et al. 2009).

The significant effect of factor interactions between sodium oleate and pH was relevant for a poorly soluble and ionisable drug. Sodium oleate is known for improving the solubility and dissolution of poorly soluble drugs but is most commonly used in the fed state since it is a digestion component. Nevertheless the presence of sodium oleate in fasted biorelevant media has been introduced in the latest fasted simulated intestinal fluid version (FaSSIF-V3) but only a few studies have included sodium oleate in their fasted biorelevant media to forecast the solubility of poorly soluble drugs (Cristofoletti & Dressman 2016; Fuchs et al. 2015; Zhou et al. 2017a). The significant factor interactions between lecithin and buffer means that the combination of those two factors decreased the solubility of AT13148. Similar features were observed in the previously published fasted and fed design of experiments. In the fasted DoE the drug aprepitant exhibited the same pattern with a significant influence of lecithin with buffer while for the fed DoE buffer and lecithin were the factors the most involved in significant interactions (Khadra et al. 2015; Zhou et al. 2017b).

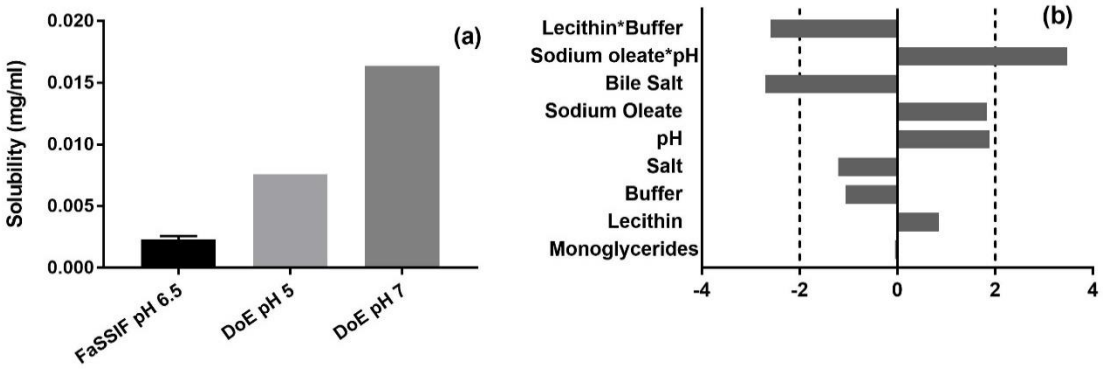

Figure 31 - Biorelevant solubility study of AT13148. (a) Mean observed biorelevant solubility in FaSSIF and DoE media, (b) Statistically standardised effect values for individual factors and factor interactions of the design of experiment. NB: For the factor interactions only the significant factors are displayed. Legend: (x-axis) standardised effect values on equilibrium solubility, vertical dashed lines indicate a statistical significance (p-value < 0.05), bar direction indicates positive or negative effect on equilibrium solubility, bar length indicates magnitude of the effect.

5.3.2. Observed clinical PK

The pharmacokinetic profiles of AT13148 for each dose level are presented in Figure 32a-b and show that maximum concentration (C_{max}) and drug exposure (AUC) were increasing with the dose. The main outcomes of the PK study was the high inter-individual variability observed at each dose level and long half-lives. The relationship between maximum concentration (C_{max}) and systemic exposure (AUC) to AT13148 dose are presented in Figure 32c-d and although the drug exhibited a large inter-individual variability at every dose level a linear relationship between the drug exposure (AUC) and the dose was concluded given the caculated r-squared value of 0.97 ($R^2 = 0.97$). Furthermore it was reported in the clinical study that a dose linearity appeared at 40mg since a 2-fold increase was observed compared to the previous dose of 20mg. The dose dependent behavior was then confirmed at 80mg since a 2-fold increase in drug exposure was again observed between dose levels.

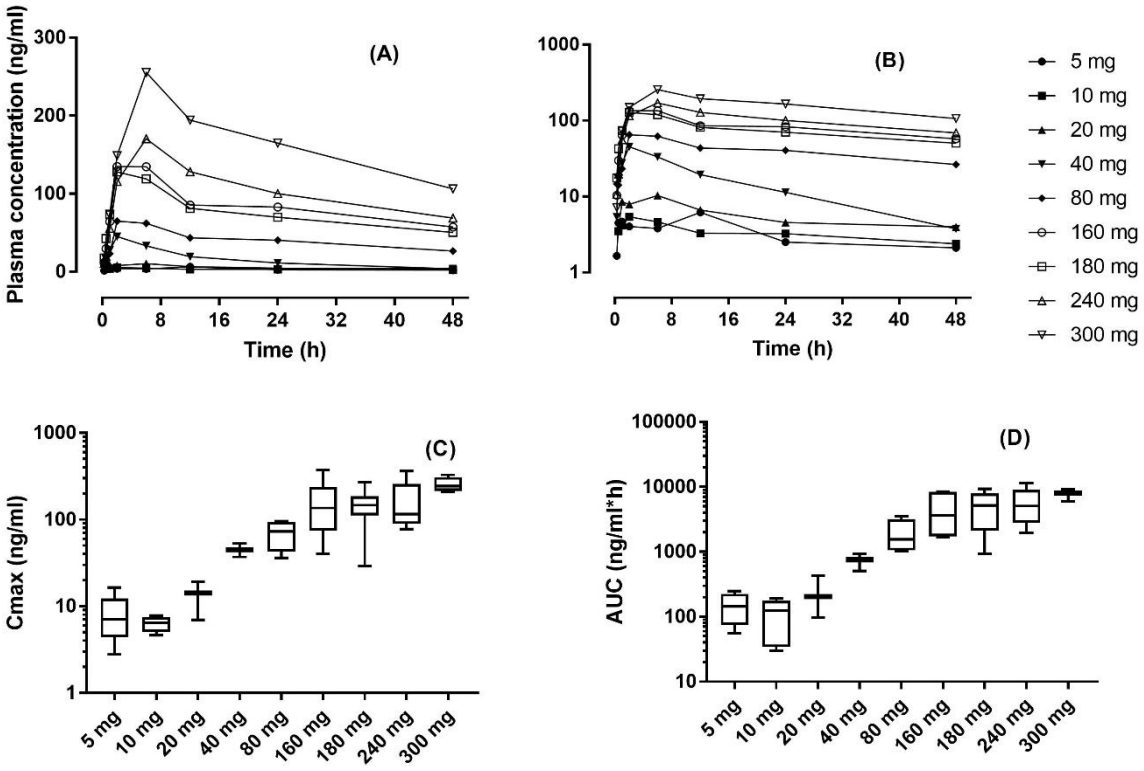

Figure 32 - Pharmacokinetic data of the clinical trial study of AT13148 given to cancer patients as increasing oral doses. (A) Plasma concentration profiles of AT13148 at each dose level on a linear scale, (B) Plasma concentration profiles of AT13148 at each dose level on a semi-logarithmic scale, (C) Boxplots of the maximum concentration (C_{max}) as a function of the dose, (D) Boxplots of the area under the plasma concentration curve (AUC) as a function of the dose.

5.3.3. PBPK modelling

The prediction of the plasma concentration profile versus time of AT13148 at 10mg dose is presented in Figure 33. The model was able to predict the plasma concentration profile of the drug at this dose level. The comparison with the mean observed clinical PK at a 10mg dose confirmed the ability of the model to predict the absorption and exposure of the drug since the predicted PK parameters maximum concentration (Cmax), time to maximum concentration (Tmax) and area under the plasma curve (AUC) were within the 20 % difference criteria (Table 25). The disposition of the drug was not fully validated since the mean predicted half-life was overpredicted with a value of 135 hours against 73 hours for the mean observed data (%difference = +85.6) (Table 25). Despite this discrepancy the model was further applied to predict the other doses of the clinical study.

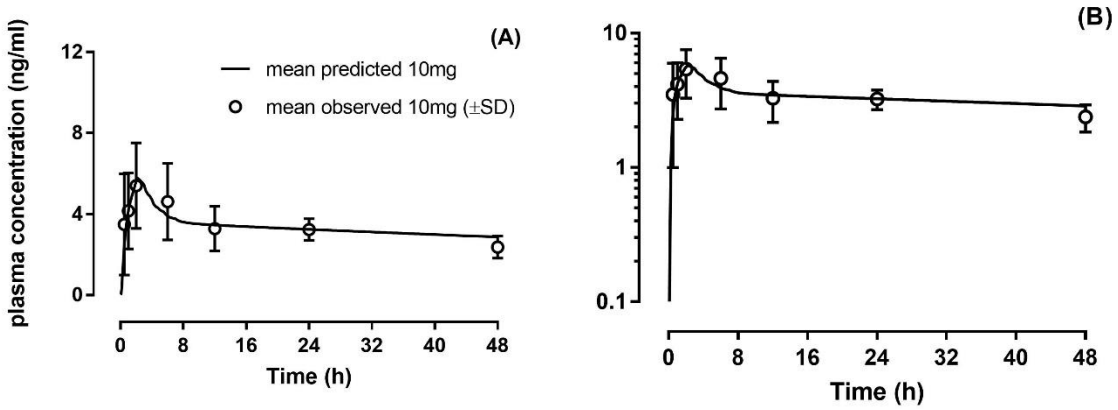

Figure 33 - Mean predicted and observed plasma concentration profile as a function of time of the drug AT13148 in cancer patients at a 10mg oral dose. (A) Linear plot of the concentration profile, (B) Semi-logarithmic plot of the concentration profile

The predictions of the plasma concentration profiles at 5 mg and 20 mg doses are presented in Figure 34. The ability to accurately predict the PK profiles was again verified against the mean observed profiles and PK parameters. The model did not accurately predict the dose escalation to 20 mg nor the dose de-escalation to 5 mg since when compared to the observed PK profiles and PK parameters it is clear that they stand outside the limit criteria (Table 25). The 20 mg dose was overpredicting the PK while the 5 mg dose was underpredicting the PK. The further doses are not displayed as the model could not be fully validated to accurately predict the different doses. During the development of the model high inter-individual variability was observed with large predicted standard deviations (Table 25). The predicted PK profiles were erratic with some virtual subjects showing flat profiles and some others showing very large maximum concentration values. Interestingly this tendency was also

highlighted in the clinical study with a consistent high variability between patients at every dose level and some patients exhibiting flat plasma profiles at some dose levels. Cancer patients are quite often on proton pump inhibitors, which means their stomach pH is not as low as a healthy person. The pH of the stomach will significantly influence whether the drug precipitates out of the PEG once the capsule has dissolved and could explain the large variability in patient profiles.

The very low predicted fraction absorbed and bioavailability (F and BA <0.03 at a 10 mg dose) of AT13148 were the main obstacles to a consistent pharmacokinetic profile and increasing the predicted dose led to even lower values of absorption (data not shown). The fraction absorbed and bioavailability were not determined in the clinical study therefore it was not possible to compare them. In order to address the limited absorption, the GIT physiological parameters and particularly the transit time in the different intestinal compartments were changed to the GIT parameters defined in the ACAT model (Agoram et al. 2001). The absorption model in PK-Sim® applies a gastric emptying time of 15 minutes with a stomach pH of 2 and a small intestine transit time of 2.10 hours. In comparison the ACAT absorption model used in GastroPlus™ (Agoram et al. 2001) defines the same gastric emptying time but with a pH of 1.3 and a longer small intestine transit time of 3.23 hours. Thererfore changing the GIT parameters allowed a longer transit time of the drug in the small intestine to be absorbed however no improvement of the fraction absorbed was observed (data not shown).

Table 25 Comparison of the predicted and observed pharmacokinetic parameters of AT13148 in cancer patients at 5, 10 and 20mg oral doses

Dose	PK parameter	Predicted (mean ± SD)	Observed (mean)	Percentage difference (%)
10 mg	C_{max} (ng/ml)	5.7 ± 4.0	5.4	+6
	T_{max} (h)	2.2 ± 0.6	2.0	+12
	$AUC_{0\text{-}tlast}$ (ng/ml*h)	161.4 ± 109.6	157.6	+2
	Half-life (h)	135.8 ± 19.6	73.0	+85
5 mg	C_{max} (ng/ml)	3.2 ± na	6.2	- 48
	T_{max} (h)	2.0 ± 0.9	12.0	- 84
	$AUC_{0\text{-}tlast}$ (ng/ml*h)	71.9 ± na	160.5	- 55
	Half-life (h)	109.0 ± na	26.0	+320
20 mg	C_{max} (ng/ml)	25.3 ± 34.9	10.3	+146
	T_{max} (h)	1.7 ± 0.5	6.0	- 72
	$AUC_{0\text{-}tlast}$ (ng/ml*h)	468.2 ± 652.5	268.5	+74
	Half-life (h)	131.3 ± 12.8	34.4	+281

SD = standard deviation, C_{max} = maximum concentration, $AUC_{0\text{-}tlast}$ = area under the curve from time zero to last concentration point, T_{max} = time to reach maximum concentration, percentage difference = (predicted – observed) / observed *100

The selected distribution model (PK-Sim standard) predicted reasonably well the processes of absorption and distribution (Figure 33). Indeed the absorption phase of the drug was well captured with acceptable predicted C_{max}, T_{max} and AUC values. However the drug disposition was not correctely estimated with a predicted sharp peak of absoption followed by a two compartment distribution phase with a rapid decay first and then a slow elimination phase which resulted in an overestimation of the half-life parameter (Table 25). When analysing the observed clinical PK profiles at the different doses (Figure 32), AT13148 did not indicate such a shape in the absorption and elimination behaviour illustrating the difficulty to correctly model the ADME properties of this class of drugs. For this class of drugs (BCS IV) the limited oral absorption is dominated by the ratio dose/solubility (Butler & Dressman 2010). Generally

these drugs will have most likely a dose dependent bioavailability with the lowest doses showing higher extent of oral absorption.

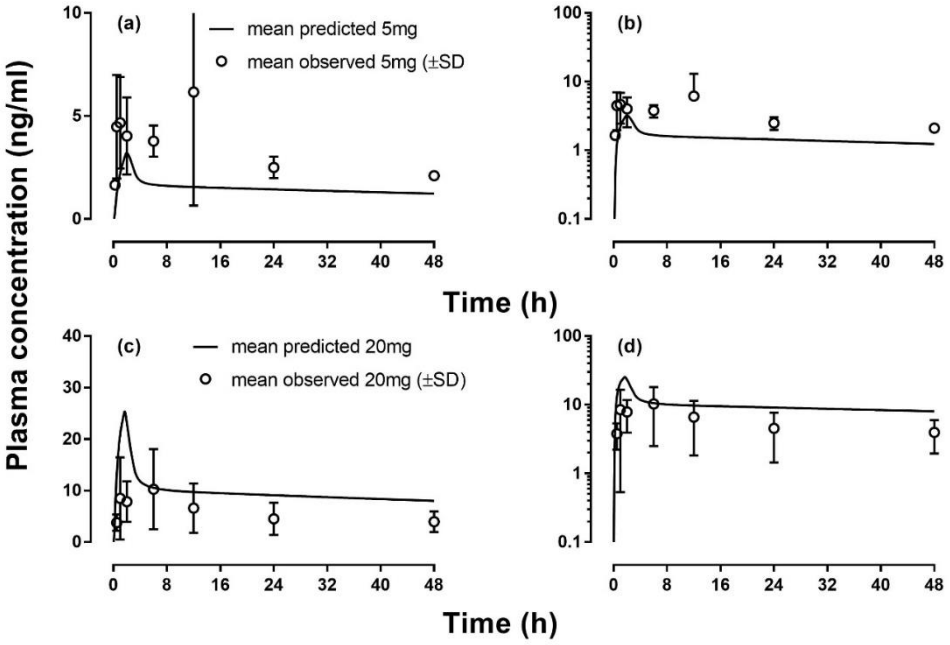

Figure 34 - Mean predicted and observed plasma concentration profile as a function of time of the drug AT13148 in cancer patients at an oral dose of 5mg (top graphics) and 20mg (bottom graphics) oral dose. (A) and (C) are plotted on a linear scale, (B) and (D) are plotted on a semi-logarithmic scale

Improving the dissolution with the use of solubilisation techniques such as micronisation or nanosuspensions to enhance dissolution and bioavailability would generally not be relevant for BCS IV drugs as reported by Butler et al. (Butler & Dressman 2010). In this model the drug was dissolved and administered as an oral capsule formulation. Yet it has been demonstrated that nanosizing and nanosuspensions can lead to even greater enhancement of bioavailability compared to micronisation with good example of marketed drugs belonging to BCS class II and IV administered orally as a nanosuspensions (Merisko-Liversidge et al. 2003; Kesisoglou & Mitra 2012). Interestingly for the formulation of AT13148 this approach could potentially mitigate the inconsistent pharmacokinetics of AT13148.

A sensitivity analysis was applied on the C_{max} and $AUC_{0\text{-}tlast}$ with respect to all the input parameters. The principle of this analysis in PK-Sim is to apply a 10% increase of all input parameters independently to be able to highlight the most influencing ones on the selected PK parameters of the drug. The results of the analysis are presented in Figure 35. Only the parameters responsible for 90% of the variations are displayed. The results are dimensionless, for instance a sensitivity of +0.5 means that a 10% increase of the parameter

will result in a 5% increase of the PK parameter. Interestingly the parameters having the most important influence on both the C_{max} and $AUC_{0\text{-}tlast}$ of AT13148 are the input solubility, the transporter ABCB1 input parameters and the first pKa value. Logically drug solubility was the main influencing parameter since according to the results of the analysis a 10% increase of the input solubility resulted in a 62% and 61% increase of C_{max} and $AUC_{0\text{-}tlast}$ respectively (Figure 35). The influence of the pKa value was of 12.7% and 13.2% variation on Cmax and $AUC_{0\text{-}tlast}$ respectively. Regarding the effect of the first pKa value a 10% increase (*i.e.* a pKa value of 1.8 instead of 1.64) is related to the pH-dependent solubility of the drug suggesting that some absorption was taking place in the stomach. As a consequence at a pH value of 2 (default fasted stomach pH value in PK-Sim®) more fraction of the drug would be non-ionised. The expression of the transporter ABCB1 (P-gp) and its concentration had a negative effect on the PK parameters Cmax and AUC which is in agreement with its role in cancer therapy by limiting the entrance of drugs in the cells (Leonard 2003).

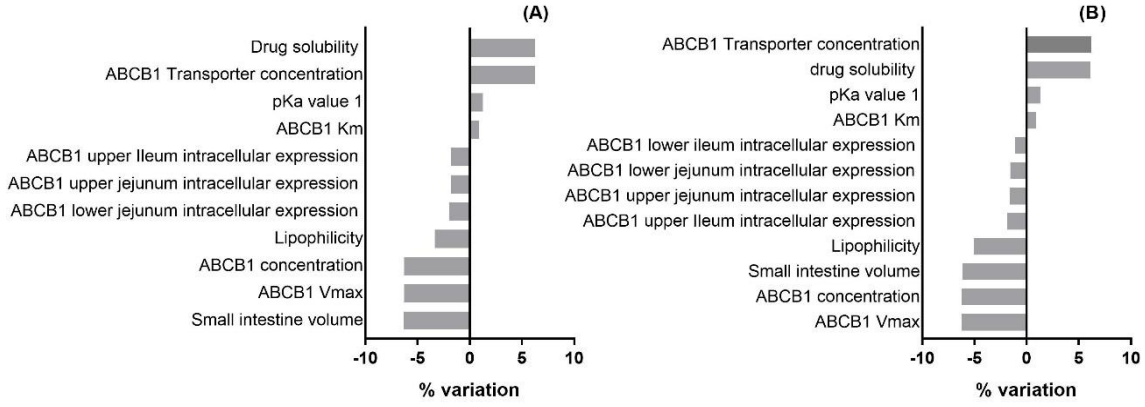

Figure 35 - Sensitivity analysis of all the model input parameters with a 10% increase on the predicted (A) maximum concentration (Cmax) and (B) area under the plasma curve (AUC). Only the parameters responsible for 90% of the variation are displayed. Sensitivities are dimensionless, for instance a sensitivity of +0.5 means that a 10% increase of the parameter leads to a 5% increase of the PK parameter

Very few PBPK models of poorly soluble and poorly permeable drugs were published in the literature (Emami Riedmaier et al. 2018; Tistaert et al. 2018). In fact for this class of drugs PBPK modelling approaches are facing the difficulty of the formulation design and the complexity of the fraction absorbed in the intestine. One study reported a successful PBPK model of venetoclax, a BCS IV drug used in the treatment of haematological malignancies such as chronic leukemia (Emami Riedmaier et al. 2018). The model was validated and used

to predict the observed food effect on healthy volunteers along with drug interactions with a CYP3A inhibitor and inducer. The crystalline form of this drug has a solubility of less than 4 ng/ml thus the solubility limitation was surmonted by an amorphous solid dispersion (ASD). The use of the amorphous form of poorly soluble drugs is known for exhibiting higher water solubility and enhanced epithelial permeability (Newman et al. 2012). The amorphous solid dispersion method is a successful formulation for drugs with high molecular weight since the principle is to generate a sustainable supersaturated formulation with the amorphous form and drugs with higher molecular weight showed more stability in physiological conditions (Raina et al. 2015).

Another study reported a successful PBPK model of the BCS IV drug ribociclib (Tistaert et al. 2018). Unlike AT13148 this basic drug exhibits an important pH-dependent solubility in phosphate buffer with solubility greater than 2.4 mg/ml at pH below 4.5 and a similar profile in biorelevant fasted and fed media. As a consequence the modelling demonstrated that the absorption rate of ribociclib was not depending on its solubility and dissolution thus no specific formulation was necessary for an *in vivo* administration. The use of PBPK modelling to predict the processes of absorption, distribution and elimination of AT13148 was not successful. The model development would require further *in vitro* investigation to achieve better results. For example permeability studies should identify and quantify the transporter protein responsible for the efflux of the drug and the formulation of the drug could be addressed. The PBPK modelling approach became very appealing over the last 10 years since it only requires *in vitro* data as input parameters and it could eventually replace animal and human studies. However it may also be restricted by the lack of sufficient *in vitro* and *in vivo* data to fully understand and model the processes of the studied drugs (Zhuang & Lu 2016).

5.4. Conclusion

The PBPK modelling approach has been quite extensively used and published particularly for poorly soluble drugs (BCS II) to predict the observed food effect (Li et al. 2018) and formulation studies whereas the models investigating the pharmacokinetic of BCS IV drugs are very sparse. The oral absorption of drugs belonging to this class will generally be driven by the ratio dose/solubility since the dissolution in the intestinal fluid will be constrained by a poor solubility and bioavailability will be dose dependent with lowest doses showing higher extent of absorption. On the other hand poorly water-soluble drugs profiting from a significant biorelevant solubilising effect or ionisable drugs showing an important pH-solubility profile will managed to achieve adequate bioavailability. Poor bioavailability commonly results in higher *in vivo* PK variability which can lead to the use of higher doses to determine clinical safety threshold or even to reach the desired pharmacological response.

In this study the cancer drug AT13148 was investigated and a PBPK modelling approach was utilised to predict its absorption, distribution, metabolism and disposition in a customised cancer population. The objective of the study was to recreate *in silico* the phase I clinical trial which consisted of a dose escalation study to evaluate the safety, efficacy and recommended dose of AT13148. Due to its poor solubility and permeability and the sparse *in vitro* data, the model development of AT13148 was expected to be very challenging. Caco-2 permeability data were available. The values showed that apparent permeability (P_{app}) from apical to basolateral (A-B) and from basolateral to apical (B-A) were 0.48 and $3.58x10^{-6}$ cm/s respectively. The results confirmed the low permeability of the drug (P_{app} A-B lower than $1x10^{-6}$ cm/s) and in addition the efflux ratio of 7.4 (P_{app} B-A / A-B greater than 2) suggests that AT13148 may be substrate of an active efflux transporter. Further investigations on the identification and quantification of the efflux perpetrator were not available. Despite its solubility and permeability profile the physicochemical properties were favourable for an oral formulation with a molecular weight lower than 500 g/mol and a moderate liphphilicity (logP = 2.16). Regarding the solubility it was determined in two biorelevant media and the DoE media produced a better solubilising effect since the resulted mean equilibrium solubility was higher compared to the FaSSIF media (Figure 31). Yet the PBPK model developed in PK-Sim® could not be fully validated. In fact the PK parameters C_{max}, T_{max} and $AUC_{0\text{-}tlast}$ at the 10 mg dose level were accurately predicted however the predicted half-life was overpredicted (Table 25). Unsurprisingly the main obstacle in the model was the absorption of the drug in the intestinal tract.

The drug was given as a dissolved formulation in capsules therefore it had to permeate through the intestinal epithelium but the predicted fraction absorbed was very low (F = 0.03 at 5 mg and 10 mg) and this value was even lower when dose was increased. According to preclinical studies, the bioavailability was good indicating that permeability was not an issue. As a consequence the conclusion could be that the formulation was suboptimal and did not address the potential for precipitation with the low aqueous solubility of AT13148.

Interestingly high inter-individual variabilities were predicted by the model and this is line with the observed clinical study where high variability was noticed at each dose level.

The parameter estimation tool implemented in PK-Sim® was employed in order to optimise the permeabililty value along with the liver intrinsic clearance since the permeability was calculated and the clearance was experimentally determined in the post mitochondrial fraction of liver. Since no significant improvement of the prediction was noted a sensitivity analysis was further performed. The sensitivity analysis tool highlighted the input parameters yielding the most significant influence on the predicted pharmacokinetics of the drug. The influence on the maximum concentration (C_{max}) and area under the plasma curve ($AUC_{0\text{-}tlast}$) were

looked at and drug input solubility, efflux transporter and first pKa value were the parameters involved in the most important PK parameter variations. In order to address this unsuccessful model more *in vitro* information on the drug would be a further requirement such as a specific study of the transporter involved in the observed efflux process. In addition a suggestion was made with respect to the formulation of the drug. Indeed the drug AT13148 could benefit from a nanosuspension formulation to enhance dissolution and as a consequence bioavailability since it has been demonstrated that a BCS IV drug could benefit from this method.

According to the observed clinical study the drug exposure was increasing with the dose suggesting that the model was clearly missing a process involved. The capacity of the PK-Sim® model to successfully predict this drug could be challenged by another PBPK software for comparison. However given the physico-chemical properties of this drug its behaviour is very likely to be drug related and not model related. This study emphasised the difficulty of developing BCS IV drugs as well as using a PBPK approach for this class of drugs. On the other hand the utility of PBPK modelling has demonstrated interesting successful results and is rapidly growing. In the field of oncology a recent publication reviewed 40 PBPK studies published between 1980 and 2017 and the different types of studies included were first-in-human studies, phase II/III trials, organ impairment study, paediatrics and drug-drug interaction studies(Saeheng et al. 2018). The results show that all the models were successfully falling within an accepted range of 1.3-fold difference but as mentioned in this review the publication of unsuccessful PBPK models could be in the interest of every researcher to move towards a better understanding of the modelling approach (Saeheng et al. 2018).

Chapter 6

General conclusion and perspectives

6. General conclusion and further perspectives

This work has been focused on the characterisation of drug solubility in human intestinal fluid and the different challenges caused by its complexity. The aim was to obtain a better understanding of the human intestinal fluid to be able to forecast and anticipate the *in vivo* human absorption. Interestingly the biorelevant media developed and presented herein has demonstrated the possibility to investigate the fasted and fed intestinal fluid and obtain key factors controlling the solubility of poorly soluble drugs in a sensible experiment confirming the relevance of such media. The use of biorelevant solubility and dissolution media has now been largely acknowledged in the pharmaceutical process of development and particularly for poorly soluble drugs belonging to class II and IV of the BCS classification. In fact water-solubility is a requirement for oral drugs and the extensive growing of the use of simulated gastric and intestinal fluids over the past 20 years. The physicochemical characteristics of such drugs has demonstrated interesting pharmacological effect on potential therapeutic targets however they become very challenging when developing an oral formulation. Various techniques have been developed and exploited to address this problem however they all provide advantages and limitations. In this study the media developed included the most important components found in the human intestinal fluid and this method demonstrated successful results by its ability to simulate solubility space comparable to previous separated studies in the fasted and fed state and also comparable to the standard FaSSIF and FeSSIF media. In addition the statistical design of experiment approach could inform on the components showing a significant influence on the solubility with relevant trends since the acidic drugs exhibited a pH-dependent solubility and neutral drugs a surfactant driven effect. The development of this new biorelevant media was driven by the need to better understand intestinal solubility which remains complex and could benefit from a more sensible estimation to reflect the variability observed *in vivo*. However the number of measurements suggested in this study limits the application in standard large scale screening therefore the minimum and maximum values were proposed. The improvement of biorelevant media has increased constantly over the past 20 years as reported by Bou-Chacra and colleagues (Bou-Chacra et al. 2017) and it will keep evolving with the knowledge on the human intestinal fluids.

Drug formulation was then investigated with the application of the Developability Classification System (DCS) and the advantage of this method was demonstrated with poorly soluble drugs exhibiting various ionisation properties. This method is particularly profitable for poorly soluble and highly permeable drugs (BCS II) due to the compensatory effect of those two parameters as described by Butler and colleagues in their work on the DCS classification. On the other hand the limitations of this approach were highlighted. For instance the comparison

of a single FaSSIF solubility with the variability observed with the new biorelevant media developed in this research emphasised the risk of over or underprediction of drug solubility. Yet for formulators it remains an easy and practical tool to make rational decisions on the choice of formulations for dissolution limited drugs.

The computational prediction of biorelevant solubility showed very promising features. Forecasting the expected *in vivo* solubility using computer models is challenging but it could be a significant support for early stage development. The predictions presented in this study achieved satisfactory results for the acidic group and for drugs in the fed state however it showed some limitations to accurately predict the neutral drugs and drugs in the fasted state. During this research project this approach was considered for its potential to reduce the need for *in vitro* studies and also because the number of computer models integrating prediction tools confirms the trend toward more model based drug development. However the mechanistic models seem to lack sensitivity. Drug research and development is moving gradually toward more powerful and comprehensive mechanistic models and it is a promising perspective for solubility and dissolution studies. Two models have been studied herein but further test studies on other computer models such as the ADMET predictor would be the next phase following this research project.

Similarly the physiologically based pharmacokinetic approach is a computational technique. This technique is building mechanistically the human and animal physiology in order to forecast the *in vivo* behaviour of drugs. The aim of such studies is to reduce and eventually to replace *in vivo* studies on animal or human. Currently the use of PBPK modelling in drug development is extensively used for different applications such as the investigation of drug interactions, food effect studies and adaptation of dose to specific populations. It has already proven very successful results, therefore the PBPK approach is now accepted by the regulatory authorities under specific conditions. During this research project the use of PBPK modelling was first utilised for the pharmacokinetic prediction of well-known drugs and the predictions were satisfactory. PBPK modelling was also used to study the direct effect of input solubility on the exposure of poorly soluble drugs. Interestingly for two drugs changing the input solubility produced significant variations of the drug exposure but without clinical consequences. As a consequence it could be of interest to forecast the *in vitro* solubility and *in vivo* plasma profile with at least three values of biorelevant solubility.

Contrastingly when the PBPK method was applied to a newly developed drug and especially a cancer drug it presented some limitations. However it should be noted that the problems were mostly drug related since the molecule belongs to the BCS class IV and exhibited a very low solubility and permeability. The PBPK models always simulate in a proportional fashion unless specified by a saturable process in the absorption or elimination. In the case of the

cancer molecule studied here the observed clinical data (C_{max} and AUC) were reported proportional to the dose administered meaning that despite the limited permeability the model was obviously missing a process which would have circumved this limitation.

This is one of the main outcomes of this study. Sufficient water-solubility is an essential parameter for drugs to be administered orally and to achieve a consistent pharmacokinetic profile. For poorly soluble drugs an enhancement is generally observed but the extent of the micelle solubilisation is specific to each drug and depends not only on the pKa and lipophilicity but as demonstrated herein on multifactorial aspects.

Since its emergence in the 1980s the development and application of PBPK modelling has significantly grown and particularly over the last 15 years. The use of this approach has demonstrated appealing and successful application in the organisation and integration of mechanistic data to create hypothesis, conduct new experimental studies, characterise pharmacokinetic variabilities and also extrapolate across species or even interpret epidemiologic studies. However this growing interest seems almost too rapid because important challenges remain and can be listed in three categories, (i) challenges in model reviewing (ii) challenges in model extrapolation and (iii) challenges in model transfer across platforms. The first challenge is related to regulatory authorities and decision-making since there is currently a lack of sufficient qualified individuals for leading appropriate model development. In addition the lack of sufficient expert reviewers along with standardised reviewing or submission processes in PBPK modelling leads to inadequate evaluation of conclusions based on a PBPK approach. Further when building a PBPK model it is common to extrapolate from *in vitro* and *in vivo* animal data or even in vitro human data to predict human models. However if no human *in vivo* data are available to compare the predicted versus observed then the challenge of the confidence in the prediction in human emerges since there is no rational ground to draw solid conclusions. In fact published papers containing no human observed data are generally academic exercises to understand specific mechanisms whereas when submitting to authorities a validation on human data is mandatory. The third challenge is regarding the choice of the modelling platform because the increasing number of modelling softwares becomes an issue. This large number of platforms becomes an obstacle for reviewers who require extensive knowledge on different software, this is why authorities such as the FDA and EMA currently consider applications from Simcyp® and GastroPlus®.

The computational modelling approach is growing rapidly with more and more sophisticated models to improve its parametrisation and application. The challenges discussed here should not be an obstacle for improving the research and development of new medicines and better communication between model developers, research investigators and authorities would be

in the interest of all. In addition, the increasing number of PBPK publications, if transparent enough on the models and techniques employed, will significantly help improve the knowledge in model based drug development.

7. References

Abuhelwa, A.Y. et al., 2017. Food, gastrointestinal pH, and models of oral drug absorption. *European Journal of Pharmaceutics and Biopharmaceutics*, 112, pp.234–248. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0939641116305756 [Accessed July 18, 2017].

Agoram, B., Woltosz, W.S. & Bolger, M.B., 2001. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Advanced drug delivery reviews*, 50 Suppl 1, pp.S41–S67.

Alam, M.A., Al-Jenoobi, I. & Al-Mohizea, A.M., 2011. Everted gut sac model as a tool in pharmaceutical research: limitations and applications. Available at: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.2042-7158.2011.01391.x [Accessed August 17, 2018].

Alibhai, S.M.H. & Horgan, A.M., 2011. Cancer screening in older adults: What to do when we don't know. *Journal of Geriatric Oncology*, 2(3), pp.149–160. Available at: https://www.sciencedirect.com/science/article/pii/S1879406811000270 [Accessed August 23, 2018].

Alsenz, J. & Kansy, M., 2007. High throughput solubility measurement in drug discovery and development. *Advanced Drug Delivery Reviews*, 59(7), pp.546–567.

Alván, G. et al., 1975. Pharmacokinetics of indomethacin. *Clinical Pharmacology and Therapeutics*, 18(3), pp.364–373.

Amidon, G.L. et al., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical research*, 12(3), pp.413–20. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7617530 [Accessed December 9, 2016].

Ashby, L.J., Beezer, A.E. & Buckton, G., 1989. In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. I. Exploration of alternative methodology: microcalorimetry. *International Journal of Pharmaceutics*, 51(3), pp.245–251. Available at: http://www.sciencedirect.com/science/article/pii/0378517389901981 [Accessed December 11, 2015].

Attwood, D. & Florence, A.T., 1983. Pharmaceutical aspects of solubilization. In *Surfactant Systems*. Dordrecht: Springer Netherlands, pp. 293–387. Available at: http://www.springerlink.com/index/10.1007/978-94-009-5775-6_6 [Accessed July 21,

2017].

Augustijns, P. et al., 2014. A review of drug solubility in human intestinal fluids: Implications for the prediction of oral absorption. *European Journal of Pharmaceutical Sciences*, 57(1), pp.322–332.

Avdeef, A. et al., 1998. pH-metric logP 10. Determination of liposomal membrane-water partition coefficients of ionizable drugs. *Pharmaceutical research*, 15(2), pp.209–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9523305 [Accessed July 23, 2018].

Avdeef, A., 1998. pH-metric Solubility. 1. Solubility-pH Profiles from Bjerrum Plots. Gibbs Buffer and pKa in the Solid State.pdf. *Pharm. Pharmacol. Commun.*, 4, pp.165–178.

Avdeef, A., Berger, C.M. & Brownell, C., 2000. pH-metric solubility. 2: correlation between the acid-base titration and the saturation shake-flask solubility-pH methods. *Pharmaceutical research*, 17(1), pp.85–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10714613 [Accessed July 6, 2017].

Axelson, J.E. et al., 1987. Food increases the bioavailability of propafenone. *British journal of clinical pharmacology*, 23(6), pp.735–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3606933 [Accessed August 18, 2018].

Baka, E., Comer, J.E.A. & Takács-Novák, K., 2008. Study of equilibrium solubility measurement by saturation shake-flask method using hydrochlorothiazide as model compound. *Journal of Pharmaceutical and Biomedical Analysis*, 46(2), pp.335–341. Available at: https://www.sciencedirect.com/science/article/pii/S0731708507006334#sec1 [Accessed August 17, 2018].

Bakatselou, V., Oppenheim, R.C. & Dressman, J.B., 1991. Solubilization and wetting effects of bile salts on the dissolution of steroids. *Pharmaceutical research*, 8(12), pp.1461–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1808607 [Accessed July 20, 2016].

Bardelmeijer, H.A. et al., 2000. The oral route for the administration of cytotoxic drugs: strategies to increase the efficiency and consistency of drug delivery. *Investigational new drugs*, 18(3), pp.231–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10958591 [Accessed July 13, 2017].

Barry, B.W. & El Eini, D.I., 1976. Solubilization of hydrocortisone, dexamethasone, testosterone and progesterone by long-chain polyoxyethylene surfactants. *The Journal of pharmacy and pharmacology*, 28(3), pp.210–8. Available at:

http://www.ncbi.nlm.nih.gov/pubmed/6695 [Accessed July 21, 2017].

Beierle, I., Meibohm, B. & Derendorf, H., 1999. Gender differences in pharmacokinetics and pharmacodynamics. *International journal of clinical pharmacology and therapeutics*, 37(11), pp.529–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10584975 [Accessed July 13, 2017].

Benet, L.Z., 2013. The role of BCS (biopharmaceutics classification system) and BDDCS (biopharmaceutics drug disposition classification system) in drug development. *Journal of pharmaceutical sciences*, 102(1), pp.34–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23147500 [Accessed June 28, 2017].

Berezhkovskiy, L.M., 2010. A Valid Equation for the Well-Stirred Perfusion Limited Physiologically Based Pharmacokinetic Model that Consistently Accounts for the Blood–Tissue Drug Distribution in the Organ and the Corresponding Valid Equation for the Steady State Volume of Distribution. *Journal of Pharmaceutical Sciences*, 99(1), pp.475–485. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19492340 [Accessed April 9, 2019].

Bergström, C. a S. et al., 2010. Dissolution Rate and Apparent Solubility of Poorly Soluble Compounds in Biorelevant Fluids. , 5(5), p.1801.

Bergström, C.A.S. & Larsson, P., 2018. Computational prediction of drug solubility in water-based systems: Qualitative and quantitative approaches used in the current drug discovery and development setting. *International Journal of Pharmaceutics*, 540(1–2), pp.185–193. Available at: https://doi.org/10.1016/j.ijpharm.2018.01.044.

Bermejo, M. et al., 2004. PAMPA—a drug absorption in vitro model. *European Journal of Pharmaceutical Sciences*, 21(4), pp.429–441. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0928098703002811 [Accessed July 12, 2017].

Bevernage, J. et al., 2010. Drug Supersaturation in Simulated Human Intestinal Fluids Representing Different Nutritional States. *Journal of Pharmaceutical Sciences*, 99(11), pp.4525–4534.

Boni, J.E., Brickl, R.S. & Dressman, J., 2007. Is bicarbonate buffer suitable as a dissolution medium? *The Journal of pharmacy and pharmacology*, 59(10), pp.1375–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17910812 [Accessed December 10, 2015].

Bou-Chacra, N. et al., 2017. Evolution of Choice of Solubility and Dissolution Media After

Two Decades of Biopharmaceutical Classification System. *The AAPS Journal*, 19(4), pp.989–1001. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28516359 [Accessed August 15, 2018].

van Breemen, R.B. & Li, Y., 2005. Caco-2 cell permeability assays to measure drug absorption. *Expert Opinion on Drug Metabolism & Toxicology*, 1(2), pp.175–185. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16922635 [Accessed August 17, 2018].

Buckton, G. et al., 1989. In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. II. Probing drug/food interactions using microcalorimetry. *International Journal of Pharmaceutics*, 56(2), pp.151–157. Available at: http://www.sciencedirect.com/science/article/pii/0378517389900082 [Accessed December 11, 2015].

Butler, J.M. & Dressman, J.B., 2010. The Developability Classification System: Application of Biopharmaceutics Concepts to Formulation Development. *Journal of Pharmaceutical Sciences*, 99(12), pp.4940–4954. Available at: https://www.sciencedirect.com/science/article/pii/S0022354915323832 [Accessed August 2, 2018].

Cannistra, S.A., 2008. Challenges and Pitfalls of Combining Targeted Agents in Phase I Studies. *Journal of Clinical Oncology*, 26(22), pp.3665–3667. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18669449 [Accessed August 22, 2018].

Cavazzuti, M., 2013. Design of Experiments. In *Optimization Methods*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 13–42. Available at: http://link.springer.com/10.1007/978-3-642-31187-1_2 [Accessed August 21, 2018].

Chabner, B.A. et al., 1978. Methotrexate Disposition in Humans: Case Studies in Ovarian Cancer and Following High-Dose Infusion. *Drug Metabolism Reviews*, 8(1), pp.107–117. Available at: http://www.ncbi.nlm.nih.gov/pubmed/363377 [Accessed July 13, 2018].

Chakraborty, S. et al., 2009. Assessment of solubilization characteristics of different surfactants for carvedilol phosphate as a function of pH. *Journal of Colloid and Interface Science*, 335(2), pp.242–249.

Chaumeil, J.C., 1998. Micronization: a method of improving the bioavailability of poorly soluble drugs. *Methods and findings in experimental and clinical pharmacology*, 20(3), pp.211–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9646283 [Accessed July

21, 2017].

Cheeti, S. et al., 2013. A physiologically based pharmacokinetic (PBPK) approach to evaluate pharmacokinetics in patients with cancer. *Biopharmaceutics & Drug Disposition*, 34(3), pp.141–154. Available at: http://doi.wiley.com/10.1002/bdd.1830.

Choi, H.-G. et al., 2015. Enhanced oral bioavailability of fenofibrate using polymeric nanoparticulated systems: physicochemical characterization and in vivo investigation. *International Journal of Nanomedicine*, 10, p.1819. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25784807 [Accessed August 8, 2018].

Clarysse, S. et al., 2011. Intestinal drug solubility estimation based on simulated intestinal fluids: Comparison with solubility in human intestinal fluids. *European Journal of Pharmaceutical Sciences*, 43(4), pp.260–269. Available at: http://dx.doi.org/10.1016/j.ejps.2011.04.016.

Clarysse, S. et al., 2009. Postprandial changes in solubilizing capacity of human intestinal fluids for BCS class II drugs. *Pharmaceutical Research*, 26(6), pp.1456–1466. Available at: http://link.springer.com/10.1007/s11095-009-9857-7 [Accessed August 25, 2016].

Comer, J.E.A., High-Throughput Measurement of log D and pKa. In *Drug Bioavailability*. Weinheim, FRG: Wiley-VCH Verlag GmbH & Co. KGaA, pp. 21–45. Available at: http://doi.wiley.com/10.1002/3527601473.ch2 [Accessed June 29, 2017].

Cristofoletti, R. & Dressman, J.B., 2016. FaSSIF-V3, but not compendial media, appropriately detects differences in the peak and extent of exposure between reference and test formulations of ibuprofen. *European Journal of Pharmaceutics and Biopharmaceutics*, 105, pp.134–140. Available at: https://www.sciencedirect.com/science/article/pii/S0939641116302107?via%3Dihub [Accessed August 31, 2018].

Daublain, P. et al., 2017. Analyzing the Potential Root Causes of Variability of Pharmacokinetics in Preclinical Species. Available at: https://pubs.acs.org/sharingguidelines [Accessed August 15, 2018].

Dauzat, M. et al., 1994. Meal induced changes in hepatic and splanchnic circulation: a noninvasive Doppler study in normal humans. *European journal of applied physiology and occupational physiology*, 68(5), pp.373–80. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8076615 [Accessed July 19, 2017].

Davit, B.M. et al., 2016. BCS Biowaivers: Similarities and Differences Among EMA, FDA,

and WHO Requirements. *The AAPS journal*, 18(3), pp.612–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26943914 [Accessed June 29, 2017].

Dekhuijzen, P.N.R. & Koopmans, P.P., 2002. Pharmacokinetic Profile of Zafirlukast. *Clinical Pharmacokinetics*, 41(2), pp.105–114. Available at: http://link.springer.com/10.2165/00003088-200241020-00003 [Accessed August 8, 2018].

Derendorf, H. et al., 2005. Dipyridamole Bioavailability in Subjects With Reduced Gastric Acidity. *The Journal of Clinical Pharmacology*, 45(7), pp.845–850. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15951475 [Accessed August 13, 2018].

Dresse, A. et al., 1978. Human pharmacokinetics of two crystalline and galenic forms of diflunisal, a new analgesic. *Pharmaceutica acta Helvetiae*, 53(6), pp.177–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/310127 [Accessed July 21, 2017].

Dressman, J.B. et al., 1998. Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms. *Pharmaceutical Research*, 15(1), pp.11–22. Available at: http://link.springer.com/article/10.1023/A%3A1011984216775 [Accessed December 4, 2015].

Dressman, J.B. et al., 2007. Estimating drug solubility in the gastrointestinal tract. *Advanced drug delivery reviews*, 59(7), pp.591–602. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17599644 [Accessed December 20, 2015].

Dunselman, P.H.J.M. & Edgar, B., 1991. Felodipine Clinical Pharmacokinetics. *Clinical Pharmacokinetics*, 21(6), pp.418–430. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1782737 [Accessed August 8, 2018].

Edginton, A.N., Schmitt, W. & Willmann, S., 2006. Development and Evaluation of a Generic Physiologically Based Pharmacokinetic Model for Children. *Clinical Pharmacokinetics*, 45(10), pp.1013–1034. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16984214 [Accessed July 13, 2018].

Edginton, A.N. & Willmann, S., 2008. Physiology-Based Simulations of a Pathological Condition. *Clinical Pharmacokinetics*, 47(11), pp.743–752. Available at: http://www.ncbi.nlm.nih.gov/pubmed/18840029 [Accessed July 13, 2018].

Edward, J.T., 1970. Molecular volumes and the Stokes-Einstein equation. *Journal of Chemical Education*, 47(4), p.261. Available at: http://pubs.acs.org/doi/abs/10.1021/ed047p261 [Accessed April 6, 2019].

Elworthy, P.H. & Lipscomb, F.J., 1968. Solubilization of griseofulvin by nonionic surfactants. *Journal of Pharmacy and Pharmacology*, 20(11), pp.817–824. Available at: http://doi.wiley.com/10.1111/j.2042-7158.1968.tb09654.x [Accessed July 13, 2018].

Emami Riedmaier, A. et al., 2018. Mechanistic Physiologically Based Pharmacokinetic Modeling of the Dissolution and Food Effect of a Biopharmaceutics Classification System IV Compound—The Venetoclax Story. *Journal of Pharmaceutical Sciences*, 107(1), pp.495–502. Available at: https://doi.org/10.1016/j.xphs.2017.09.027.

EMEA, 2001. Note of Guidance Bioequivalence. *Reproduction*, (December 2000), pp.0–18.

Engeland, A. et al., 2005. Height and Body Mass Index in Relation to Colorectal and Gallbladder Cancer in Two Million Norwegian Men and Women. *Cancer Causes & Control*, 16(8), pp.987–996. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16132807 [Accessed August 23, 2018].

Etherson, K., Halbert, G. & Elliott, M., 2014. Determination of excipient based solubility increases using the CheqSol method. *International journal of pharmaceutics*, 465(1–2), pp.202–9. Available at: http://www.sciencedirect.com/science/article/pii/S0378517314000891.

Evans, D.F. et al., 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut*, 29(8), pp.1035–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3410329 [Accessed July 26, 2018].

Fagerberg, J.H. et al., 2015. Computational prediction of drug solubility in fasted simulated and aspirated human intestinal fluid. *Pharmaceutical research*, 32(2), pp.578–89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25186438 [Accessed July 2, 2018].

Fagerberg, J.H. et al., 2012. Ethanol Effects on Apparent Solubility of Poorly Soluble Drugs in Simulated Intestinal Fluid. *Molecular Pharmaceutics*, 9(7), pp.1942–1952. Available at: http://pubs.acs.org/doi/10.1021/mp2006467 [Accessed July 26, 2018].

Fagerholm, U. et al., 1999. Jejunal permeability in humans in vivo and rats in situ: Investigation of molecular size selectivity and solvent drag. *Acta Physiologica Scandinavica*, 165(3), pp.315–324. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10192182 [Accessed August 12, 2018].

Fagerholm, U., Johansson, M. & Lennernäs, H., 1996. Comparison between permeability coefficients in rat and human jejunum. *Pharmaceutical research*, 13(9), pp.1336–42. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8893271 [Accessed July 11, 2017].

Fallingborg, J., 1999. Intraluminal pH of the human gastrointestinal tract. *Danish medical bulletin*, 46(3), pp.183–96. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10421978 [Accessed August 20, 2018].

FDA, 2002. Guidance for Industry Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations. *FDA Guidance*, (July), pp.1–24.

FDA & CDER, 2015. Guidance for Industry, Waiver of in vivo bioavailability and bioequivalence studies for immediate release solid oral dosage forms based on a biopharmaceutics classification system. *Center for Drug Evaluation and Research*, (May), pp.1–2. Available at: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm [Accessed June 28, 2018].

Fioritto, A.F., Bhattachar, S.N. & Wesley, J.A., 2007. Solubility measurement of polymorphic compounds via the pH-metric titration technique. *International Journal of Pharmaceutics*, 330(1–2), pp.105–113. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17049191 [Accessed August 17, 2018].

Fleisher, D. et al., 1999. Drug, Meal and Formulation Interactions Influencing Drug Absorption After Oral Administration. *Clinical Pharmacokinetics*, 36(3), pp.233–254. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10223170 [Accessed August 15, 2018].

Food and Drug Administration (FDA), 2002. Food-Effect Bioavailability and Fed Bioequivalence Studies. *Guidance for Industry*, (December). Available at: https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm070241.pdf [Accessed July 13, 2018].

Forgue, S.T. et al., 2006. Tadalafil pharmacokinetics in healthy subjects. *British Journal of Clinical Pharmacology*, 61(3), pp.280–288.

Francis, S.H. & Corbin, J.D., 2003. Molecular mechanisms and pharmacokinetics of phosphodiesterase-5 antagonists. *Current urology reports*, 4(6), pp.457–465. Available at: http://link.springer.com/10.1007/s11934-003-0027-x [Accessed February 11, 2018].

Fuchs, A. et al., 2015. Advances in the design of fasted state simulating intestinal fluids: FaSSIF-V3. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 94, pp.229–240. Available at: http://www.sciencedirect.com/science/article/pii/S0939641115002477.

Fuchs, A. & Dressman, J.B., 2014. Composition and physicochemical properties of fasted-state human duodenal and jejunal fluid: a critical evaluation of the available data. *Journal of pharmaceutical sciences*, 103(11), pp.3398–411. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25277073 [Accessed December 14, 2015].

Galia, E. et al., 1998. Evaluation of Various Dissolution Media for Predicting In Vivo Performance of Class I and II Drugs. *Pharmaceutical Research*, 15(5), pp.698–705. Available at: http://link.springer.com/article/10.1023/A%3A1011910801212 [Accessed December 9, 2015].

Gerlowski, L.E. & Jain, R.K., 1983. Physiologically Based Pharmacokinetic Modeling: Principles and Applications. *Journal of Pharmaceutical Sciences*, 72(10), pp.1103–1127. Available at: https://www.sciencedirect.com/science/article/pii/S0022354915447926 [Accessed April 6, 2019].

Ghadi, R. & Dand, N., 2017. BCS class IV drugs: Highly notorious candidates for formulation development. *Journal of Controlled Release*, 248, pp.71–95. Available at: https://www.sciencedirect.com/science/article/pii/S0168365916309841?via%3Dihub#bb0020 [Accessed September 1, 2018].

GlaxoSmithKline, 2005. *Title: A Randomized, Double-Blind, Placebo Controlled, PK/PD Modeling, Multicenter Study to Compare the B1-Blocking Effects of Carvedilol Phosphate CR Capsule Formulation to COREG® Immediate Release Tablets at Steady State in Adult Patients with Essential Hypertension*, Available at: https://www.gsk-clinicalstudyregister.com/files2/gsk-105517-902-clinical-study-report-redact.pdf [Accessed July 31, 2018].

Glomme, A., März, J. & Dressman, J.B., 2007. Predicting the Intestinal Solubility of Poorly Soluble Drugs. In *Pharmacokinetic Profiling in Drug Research*. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, pp. 259–280. Available at: http://doi.wiley.com/10.1002/9783906390468.ch16 [Accessed July 23, 2018].

Godfrey, A.R., DiGiacinto, J. & Davis, M.W., 2011. Single-Dose Bioequivalence of 105-mg Fenofibric Acid Tablets Versus 145-mg Fenofibrate Tablets Under Fasting and Fed Conditions: A Report of Two Phase I, Open-Label, Single-Dose, Randomized, Crossover Clinical Trials. *Clinical Therapeutics*, 33(6), pp.766–775. Available at: http://dx.doi.org/10.1016/j.clinthera.2011.05.047.

Gramatté, T., 1994. Griseofulvin absorption from different sites in the human small intestine.

Biopharmaceutics & drug disposition, 15(9), pp.747–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7888603 [Accessed August 19, 2018].

Greiff, J.M.C. & Rowbotham, D., 1994. Pharmacokinetic Drug Interactions with Gastrointestinal Motility Modifying Agents. *Clinical Pharmacokinetics*, 27(6), pp.447–461. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7882635 [Accessed August 18, 2018].

Grove, M. et al., 2005. Bioavailability of seocalcitol I: Relating solubility in biorelevant media with oral bioavailability in rats--effect of medium and long chain triglycerides. *Journal of pharmaceutical sciences*, 94(8), pp.1830–8. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-25444464372&partnerID=tZOtx3y1 [Accessed December 15, 2015].

Gu, C.-H. et al., 2007. Predicting Effect of Food on Extent of Drug Absorption Based on Physicochemical Properties. *Pharmaceutical Research*, 24(6), pp.1118–1130. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17385020 [Accessed August 15, 2018].

Hann, M.M., 2011. Molecular obesity, potency and other addictions in drug discovery. *MedChemComm*, 2(5), p.349. Available at: http://xlink.rsc.org/?DOI=c1md00017a [Accessed July 27, 2018].

Hardy, B.G. et al., 1988. Effect of indomethacin on the pharmacokinetics and pharmacodynamics of felodipine. *British journal of clinical pharmacology*, 26(5), pp.557–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3207560 [Accessed March 19, 2018].

Hasler, W.L., 2011. Gastroparesis: pathogenesis, diagnosis and management. *Nature Reviews Gastroenterology & Hepatology*, 8(8), pp.438–453. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21769117 [Accessed August 17, 2018].

Helander, H.F. & Fändriks, L., 2014. Surface area of the digestive tract – revisited. *Scandinavian Journal of Gastroenterology*, 49(6), pp.681–689. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24694282 [Accessed August 18, 2018].

Helleberg, L., 1981. Clinical Pharmacokinetics of Indomethacin. *Clinical Pharmacokinetics*, 6(4), pp.245–258. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7249487 [Accessed July 25, 2018].

Hidalgo, I.J., Raub, T.J. & Borchardt, R.T., 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability.

Gastroenterology, 96(3), pp.736–49. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2914637 [Accessed July 11, 2017].

Holm, R., Müllertz, A. & Mu, H., 2013. Bile salts and their importance for drug absorption. *International journal of pharmaceutics*, 453(1), pp.44–55. Available at: http://www.sciencedirect.com/science/article/pii/S0378517313002913 [Accessed November 15, 2015].

ICH Expert Working, 2009. *Nonclinical Evaluation for Anticancer Pharmaceuticals S9. International Conference on harmonisation of Technical Requirements For Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline*, Available at: https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Safety/S9/Step4/S9_Step4_Guideline.pdf [Accessed December 2, 2018].

Ilardia-Arana, D., Kristensen, H.G. & Müllertz, A., 2006. Biorelevant dissolution media: Aggregation of amphiphiles and solubility of estradiol. *Journal of Pharmaceutical Sciences*, 95(2), pp.248–255. Available at: https://www.sciencedirect.com/science/article/pii/S0022354916319542 [Accessed August 2, 2018].

Irvine, J.D. et al., 1999. MDCK (Madin-Darby Canine Kidney) Cells: A Tool for Membrane Permeability Screening. *Journal of Pharmaceutical Sciences*, 88(1), pp.28–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9874698 [Accessed July 12, 2017].

J. W. Mauger, 2017. *Physicochemical Properties of Buffers Used in Simulated Biological Fluids with Potential Application for In Vitro Dissolution Testing: A Mini-review*, salt lake city. Available at: http://www.dissolutiontech.com/issues/201708/DT201708_A03.pdf [Accessed August 21, 2018].

Jamei, M., Dickinson, G.L. & Rostami-Hodjegan, A., 2009. A Framework for Assessing Inter-individual Variability in Pharmacokinetics Using Virtual Human Populations and Integrating General Knowledge of Physical Chemistry, Biology, Anatomy, Physiology and Genetics: A Tale of 'Bottom-Up' vs 'Top-Down' Recognition of Covariates. *Drug Metabolism and Pharmacokinetics*, 24(1), pp.53–75. Available at: https://www.sciencedirect.com/science/article/pii/S1347436715300823?via%3Dihub [Accessed June 26, 2018].

Jantratid, E. et al., 2008. Dissolution Media Simulating Conditions in the Proximal Human Gastrointestinal Tract: An Update. *Pharmaceutical Research*, 25(7), pp.1663–1676. Available at: http://link.springer.com/10.1007/s11095-008-9569-4.

Jones, H. & Rowland-Yeo, K., 2013. Basic concepts in physiologically based pharmacokinetic modeling in drug discovery and development. *CPT: pharmacometrics & systems pharmacology*, 2(8), p.e63. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23945604 [Accessed June 14, 2016].

Jones, H.M. et al., 2015. Physiologically based pharmacokinetic modeling in drug discovery and development: a pharmaceutical industry perspective. *Clinical pharmacology and therapeutics*, 97(3), pp.247–262.

Jones, H.M., Gardner, I.B. & Watson, K.J., 2009. Modelling and PBPK Simulation in Drug Discovery. *The AAPS Journal*, 11(1), pp.155–166. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19280352 [Accessed August 14, 2018].

Juenemann, D. et al., 2011. Biorelevant in vitro dissolution testing of products containing micronized or nanosized fenofibrate with a view to predicting plasma profiles. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V*, 77(2), pp.257–64. Available at: http://www.sciencedirect.com/science/article/pii/S0939641110002869 [Accessed December 17, 2015].

JUNGINGER, H.E., VERHOEVEN, J. & PESCHIER, L.J.C., A new in vitro model to detect interactions between controlled release dosage forms and food. *Acta pharmaceutica technologica*, 36(3), pp.155–160. Available at: http://cat.inist.fr/?aModele=afficheN&cpsidt=19294492 [Accessed December 11, 2015].

Kalantzi, L. et al., 2006. Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharmaceutical research*, 23(1), pp.165–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16308672 [Accessed December 4, 2015].

Kansy, M., Senner, F. & Gubernator, K., 1998. Physicochemical High Throughput Screening: Parallel Artificial Membrane Permeation Assay in the Description of Passive Absorption Processes. *Journal of Medicinal Chemistry*, 41(7), pp.1007–1010. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9544199 [Accessed July 12, 2017].

Katsura, T. & Inui, K.-I., 2003. Intestinal absorption of drugs mediated by drug transporters: mechanisms and regulation. *Drug metabolism and pharmacokinetics*, 18(1), pp.1–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15618714 [Accessed July 13, 2017].

Kesisoglou, F. & Mitra, A., 2012. Crystalline Nanosuspensions as Potential Toxicology and Clinical Oral Formulations for BCS II/IV Compounds. *The AAPS Journal*, 14(4), pp.677–

687. Available at: http://www.springerlink.com/index/10.1208/s12248-012-9383-0.

Khadra, I. et al., 2015. Statistical investigation of simulated intestinal fluid composition on the equilibrium solubility of biopharmaceutics classification system class II drugs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 67, pp.65–75.

Khalil, F. & Läer, S., 2014. Physiologically based pharmacokinetic models in the prediction of oral drug exposure over the entire pediatric age range-sotalol as a model drug. *The AAPS journal*, 16(2), pp.226–39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24399240 [Accessed January 31, 2018].

Kimura, T. & Higaki, K., 2002. Gastrointestinal transit and drug absorption. *Biological & pharmaceutical bulletin*, 25(2), pp.149–64. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11853157 [Accessed August 18, 2018].

Kleberg, K., Jacobsen, F., et al., 2010. Biorelevant media simulating fed state intestinal fluids: colloid phase characterization and impact on solubilization capacity. *Journal of pharmaceutical sciences*, 99(8), pp.3522–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20564382 [Accessed December 8, 2015].

Kleberg, K., Jacobsen, J. & Müllertz, A., 2010. Characterising the behaviour of poorly water soluble drugs in the intestine: application of biorelevant media for solubility, dissolution and transport studies. *The Journal of pharmacy and pharmacology*, 62(11), pp.1656–68. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21039549 [Accessed December 7, 2015].

Klein, S. et al., 2004. Media to simulate the postprandial stomach I. Matching the physicochemical characteristics of standard breakfasts. *The Journal of pharmacy and pharmacology*, 56(5), pp.605–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15142337 [Accessed December 13, 2015].

Klein, S., 2010. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. *The AAPS journal*, 12(3), pp.397–406. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2895438&tool=pmcentrez&rendertype=abstract [Accessed November 20, 2015].

Korn, E.L. et al., 2001. Clinical Trial Designs for Cytostatic Agents: Are New Approaches Needed? *Journal of Clinical Oncology*, 19(1), pp.265–272. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11134222 [Accessed August 22, 2018].

Kostewicz, E.S. et al., 2014. PBPK models for the prediction of in vivo performance of oral dosage forms. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 57, pp.300–21. Available at: http://www.sciencedirect.com/science/article/pii/S092809871300362X [Accessed December 16, 2015].

Lee, A. & Kuo, B., 2010. Metoclopramide in the treatment of diabetic gastroparesis. *Expert review of endocrinology & metabolism*, 5(5), pp.653–662. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21278804 [Accessed August 17, 2018].

Lennernäs, H. et al., 2014. Oral biopharmaceutics tools - Time for a new initiative - An introduction to the IMI project OrBiTo. *European Journal of Pharmaceutical Sciences*, 57(1), pp.292–299.

Leonard, G.D., 2003. The Role of ABC Transporters in Clinical Practice. *The Oncologist*, 8(5), pp.411–424. Available at: http://theoncologist.alphamedpress.org/cgi/doi/10.1634/theoncologist.8-5-411.

Leppert, W., 2012. The impact of opioid analgesics on the gastrointestinal tract function and the current management possibilities. *Contemporary oncology (Poznan, Poland)*, 16(2), pp.125–31. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23788866 [Accessed August 18, 2018].

Li, M. et al., 2018. Predictive Performance of Physiologically Based Pharmacokinetic Models for the Effect of Food on Oral Drug Absorption: Current Status. *CPT: pharmacometrics & systems pharmacology*, 7(2), pp.82–89. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29168611 [Accessed September 4, 2018].

Liedholm, H., Wåhlin-Boll, E. & Melander, A., 1990. Mechanisms and variations in the food effect on propranolol bioavailability. *European journal of clinical pharmacology*, 38(5), pp.469–75. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2379531 [Accessed August 18, 2018].

Lindenberg, M., Kopp, S. & Dressman, J.B., 2004. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics*, 58(2), pp.265–278. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15296954 [Accessed June 25, 2018].

Lipinski, C., 2009. American Pharmaceutical Review - Poor Aqueous Solubility – an Industry Wide Problem in Drug Discovery American Pharmaceutical Review - Poor Aqueous

Solubility – an Industry Wide Problem in Drug Discovery. , (September 2002), pp.38–40.

Lipinski, C.A., 2000. Drug-like properties and the causes of poor solubility and poor permeability. *Journal of Pharmacological and Toxicological Methods*, 44(1), pp.235–249. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11274893 [Accessed July 13, 2017].

Lipinski, C.A. et al., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 23(1–3), pp.3–25. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0169409X96004231 [Accessed July 13, 2017].

Longo, W.E. & Vernava, A.M., 1993. Prokinetic agents for lower gastrointestinal motility disorders. *Diseases of the colon and rectum*, 36(7), pp.696–708. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8348856 [Accessed August 18, 2018].

Lozoya-Agullo, I. et al., 2015. In Situ Perfusion Model in Rat Colon for Drug Absorption Studies: Comparison with Small Intestine and Caco-2 Cell Model. *Journal of pharmaceutical sciences*, 104(9), pp.3136–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25891783 [Accessed August 17, 2018].

Luo, Z. et al., 2013. Ex vivo and in situ approaches used to study intestinal absorption. *Journal of Pharmacological and Toxicological Methods*, 68(2), pp.208–216. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23831587 [Accessed August 17, 2018].

Macheras, P., Koupparis, M. & Tsaprounis, C., 1986. Drug dissolution studies in milk using the automated flow injection serial dynamic dialysis technique. *International Journal of Pharmaceutics*, 33(1–3), pp.125–136. Available at: http://www.sciencedirect.com/science/article/pii/0378517386900463 [Accessed October 13, 2015].

Majumdar, A.K. et al., 2006. Pharmacokinetics of aprepitant after single and multiple oral doses in healthy volunteers. *Journal of Clinical Pharmacology*, 46(3), pp.291–300.

Marciani, L. et al., 2010. Effects of a 5-HT3 antagonist, ondansetron, on fasting and postprandial small bowel water content assessed by magnetic resonance imaging. *Alimentary Pharmacology & Therapeutics*, 32(5), p.no-no. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20626735 [Accessed August 12, 2018].

Marciani, L. et al., 2010. Postprandial Changes in Small Bowel Water Content in Healthy Subjects and Patients With Irritable Bowel Syndrome. *Gastroenterology*, 138(2), p.469–477.e1. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19909743 [Accessed August 12, 2018].

Markopoulos, C. et al., 2015. In-vitro simulation of luminal conditions for evaluation of performance of oral drug products: Choosing the appropriate test media. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V*, 93, pp.173–82. Available at: http://www.sciencedirect.com/science/article/pii/S093964111500140X [Accessed November 20, 2015].

Masaoka, Y. et al., 2006. Site of drug absorption after oral administration: Assessment of membrane permeability and luminal concentration of drugs in each segment of gastrointestinal tract. *European Journal of Pharmaceutical Sciences*, 29(3–4), pp.240–250. Available at: https://www.sciencedirect.com/science/article/pii/S0928098706001709 [Accessed August 18, 2018].

Van Der Meer, J.W. et al., 1980. The influence of gastric acidity on the bio-availability of ketoconazole. *The Journal of antimicrobial chemotherapy*, 6(4), pp.552–4. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6253434 [Accessed August 13, 2018].

Melander, A. et al., 1977. Enhancement of the bioavailability of propranolol and metoprolol by food. *Clinical pharmacology and therapeutics*, 22(1), pp.108–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/872491 [Accessed July 19, 2017].

Melander, A., Lalka, D. & McLean, A., 1988. Influence of food on the presystemic metabolism of drugs. *Pharmacology & Therapeutics*, 38(2), pp.253–267. Available at: https://www.sciencedirect.com/science/article/pii/016372588890099X [Accessed August 18, 2018].

Merisko-Liversidge, E., Liversidge, G.G. & Cooper, E.R., 2003. Nanosizing: A formulation approach for poorly-water-soluble compounds. *European Journal of Pharmaceutical Sciences*, 18(2), pp.113–120.

Miller, D.B. & Spence, J.D., 1998. Clinical Pharmacokinetics of Fibric Acid Derivatives (Fibrates). *Clinical Pharmacokinetics*, 34(2), pp.155–162. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9515185 [Accessed July 25, 2018].

Mithani, S.D. et al., 1996. Estimation of the increase in solubility of drugs as a function of bile

salt concentration. *Pharmaceutical research*, 13(1), pp.163–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8668668 [Accessed August 1, 2018].

Morgan, T., 1994. Clinical Pharmacokinetics and Pharmacodynamics of Carvedilol. *Clinical Pharmacokinetics*, 26(5), pp.335–346. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7914479 [Accessed July 25, 2018].

Murdande, S.B. et al., 2011. Aqueous solubility of crystalline and amorphous drugs: Challenges in measurement. *Pharmaceutical Development and Technology*, 16(3), pp.187–200. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20429826 [Accessed August 20, 2018].

Naylor, L.J., Bakatselou, V. & Dressman, J.B., 1993. Comparison of the mechanism of dissolution of hydrocortisone in simple and mixed micelle systems. *Pharmaceutical research*, 10(6), pp.865–70. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8321855 [Accessed July 20, 2016].

Newman, A., Knipp, G. & Zografi, G., 2012. Assessing the performance of amorphous solid dispersions. *Journal of Pharmaceutical Sciences*, 101(4), pp.1355–1377. Available at: https://www.sciencedirect.com/science/article/pii/S0022354915316324 [Accessed September 3, 2018].

Nicolaides, E. et al., 2001. Biorelevant dissolution testing to predict the plasma profile of lipophilic drugs after oral administration. *Pharmaceutical research*, 18(3), pp.380–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11442280 [Accessed December 17, 2015].

Nielsen, P.B. et al., 2001. The effect of α-tocopherol on the in vitro solubilisation of lipophilic drugs. *International Journal of Pharmaceutics*, 222(2), pp.217–224.

Norinder, U. & Bergström, C.A.S., 2006. Prediction of ADMET Properties. *ChemMedChem*, 1(9), pp.920–937. Available at: http://doi.wiley.com/10.1002/cmdc.200600155 [Accessed July 27, 2018].

Noyes, A.A. & Whitney, W.R., 1897. THE RATE OF SOLUTION OF SOLID SUBSTANCES IN THEIR OWN SOLUTIONS. *Journal of the American Chemical Society*, 19(12), pp.930–934. Available at: http://pubs.acs.org/doi/abs/10.1021/ja02086a003 [Accessed July 20, 2017].

Okumu, A., DiMaso, M. & Löbenberg, R., 2009. Computer simulations using GastroPlus™ to justify a biowaiver for etoricoxib solid oral drug products. *European Journal of*

Pharmaceutics and Biopharmaceutics, 72(1), pp.91–98. Available at: https://www.sciencedirect.com/science/article/pii/S0939641108004396 [Accessed August 15, 2018].

Otsuka, K., Shono, Y. & Dressman, J., 2013. Coupling biorelevant dissolution methods with physiologically based pharmacokinetic modelling to forecast in-vivo performance of solid oral dosage forms. *The Journal of pharmacy and pharmacology*, 65(7), pp.937–52. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23738721 [Accessed December 17, 2015].

Parks, D.A. & Jacobson, E.D., 1985. Physiology of the splanchnic circulation. *Archives of internal medicine*, 145(7), pp.1278–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/4015279 [Accessed August 18, 2018].

Parrott, N. et al., 2011. Development of a Physiologically Based Model for Oseltamivir and Simulation of Pharmacokinetics in Neonates and Infants. *Clinical Pharmacokinetics*, 50(9), pp.613–623. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21827216 [Accessed July 13, 2018].

Parrott, N. et al., 2009. Predicting pharmacokinetics of drugs using physiologically based modeling--application to food effects. *The AAPS journal*, 11(1), pp.45–53. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19184451 [Accessed July 13, 2018].

Patel, V.S. & Kramer, W.G., 1986. Allopurinol absorption from different sites of the rat gastrointestinal tract. *Journal of pharmaceutical sciences*, 75(3), pp.275–7. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3701610 [Accessed August 19, 2018].

Pedersen, B.L. et al., 2000. A Comparison of the Solubility of Danazol in Human and Simulated Gastrointestinal Fluids. *Pharmaceutical Research*, 17(7), pp.891–894. Available at: http://link.springer.com/article/10.1023/A%3A1007576713216 [Accessed December 4, 2015].

Pedersen, B.L. et al., 1999. Dissolution of Hydrocortisone in Human and Simulated Intestinal Fluids. *Pharmaceutical Research*, 17(2), pp.183–189. Available at: http://link.springer.com/article/10.1023/A%3A1007517414200 [Accessed December 14, 2015].

Perrier, J. et al., 2018. Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs. *European Journal of Pharmaceutical Sciences*, 111(June 2017), pp.247–256. Available at: http://www.sciencedirect.com/science/article/pii/S0928098717305511#f0035 [Accessed

October 11, 2017].

Persson, E.M. et al., 2005. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharmaceutical research*, 22(12), pp.2141–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16247711 [Accessed December 7, 2015].

Petit, E. et al., 1988. Circadian rhythm-varying plasma concentration of 5-fluorouracil during a five-day continuous venous infusion at a constant rate in cancer patients. *Cancer research*, 48(6), pp.1676–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/3345535 [Accessed June 26, 2018].

Placidi, E. et al., 2010. PTH-045 Effects of an osmotic laxative on the distribution of water between the small and large intestine in humans. *Gut*, 59(Suppl 1), p.A141.1-A141. Available at: http://gut.bmj.com/lookup/doi/10.1136/gut.2009.209049f [Accessed August 12, 2018].

Poulin, P. & Theil, F.-P., 2002. Prediction of pharmacokinetics prior to in vivo studies. 1. Mechanism-based prediction of volume of distribution. *Journal of pharmaceutical sciences*, 91(1), pp.129–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11782904 [Accessed April 9, 2019].

Psachoulias, D. et al., 2011. Precipitation in and Supersaturation of Contents of the Upper Small Intestine After Administration of Two Weak Bases to Fasted Adults. *Pharmaceutical Research*, 28(12), pp.3145–3158. Available at: http://link.springer.com/10.1007/s11095-011-0506-6 [Accessed August 9, 2017].

Raina, S.A. et al., 2015. Impact of Solubilizing Additives on Supersaturation and Membrane Transport of Drugs. *Pharmaceutical Research*, 32(10), pp.3350–3364. Available at: http://link.springer.com/10.1007/s11095-015-1712-4 [Accessed September 3, 2018].

Ran, Y. & Yalkowsky, S.H., 2001. Prediction of drug solubility by the general solubility equation (GSE). *Journal of Chemical Information and Computer Sciences*, 41(2), pp.354–357. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11277722 [Accessed July 26, 2018].

Richens, A., 1979. Clinical Pharmacokinetics of Phenytoin. *Clinical Pharmacokinetics*, 4(3), pp.153–169. Available at: http://www.ncbi.nlm.nih.gov/pubmed/383353 [Accessed August 8, 2018].

Richter, J.E. & Long, J.F., 1995. Cisapride for gastroesophageal reflux disease: a placebo-

controlled, double-blind study. *The American journal of gastroenterology*, 90(3), pp.423–30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7872282 [Accessed August 18, 2018].

Riethorst, D. et al., 2015. Characterization of Human Duodenal Fluids in Fasted and Fed State Conditions. *Journal of Pharmaceutical Sciences*, p.n/a-n/a. Available at: http://doi.wiley.com/10.1002/jps.24603.

Rodgers, T. & Rowland, M., 2007. Mechanistic Approaches to Volume of Distribution Predictions: Understanding the Processes. *Pharmaceutical Research*, 24(5), pp.918–933. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17372687 [Accessed April 9, 2019].

Rosoff, M. & Serajuddin, A.T.M., 1980. Solubilization of diazepam in bile salts and in sodium cholate-lecithin-water phases. *International Journal of Pharmaceutics*, 6(2), pp.137–146. Available at: http://linkinghub.elsevier.com/retrieve/pii/0378517380900861 [Accessed July 24, 2017].

Rowland, M., Peck, C. & Tucker, G., 2011. Physiologically-based pharmacokinetics in drug development and regulatory science. *Annual review of pharmacology and toxicology*, 51, pp.45–73. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20854171 [Accessed December 16, 2015].

Ruan, L.-P. et al., 2006. Prediction of human absorption of natural compounds by the non-everted rat intestinal sac model. *European Journal of Medicinal Chemistry*, 41(5), pp.605–610. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16546303 [Accessed July 11, 2017].

Rupp, C., Steckel, H. & Müller, B.W., 2010. Solubilization of poorly water-soluble drugs by mixed micelles based on hydrogenated phosphatidylcholine. *International journal of pharmaceutics*, 395(1–2), pp.272–80. Available at: http://www.sciencedirect.com/science/article/pii/S0378517310003868 [Accessed December 10, 2015].

Saeheng, T., Na-Bangchang, K. & Karbwang, J., 2018. Utility of physiologically based pharmacokinetic (PBPK) modeling in oncology drug development and its accuracy: a systematic review. *European Journal of Clinical Pharmacology*, pp.1–12.

Sager, J.E. et al., 2015. Physiologically Based Pharmacokinetic (PBPK) Modeling and Simulation Approaches: A Systematic Review of Published Models, Applications, and Model Verification. *Drug metabolism and disposition: the biological fate of chemicals*,

43(11), pp.1823–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/26296709.

Savjani, K.T., Gajjar, A.K. & Savjani, J.K., 2012. Drug Solubility: Importance and Enhancement Techniques. *ISRN Pharmaceutics*, 2012, pp.1–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22830056 [Accessed July 19, 2016].

Saxty, G. et al., 2007. Identification of Inhibitors of Protein Kinase B Using Fragment-Based Lead Discovery †. *Journal of Medicinal Chemistry*, 50(10), pp.2293–2296. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17451234 [Accessed December 2, 2018].

Shankland, W., 1970. The equilibrium and structure of lecithin-cholate mixed micelles. *Chemistry and Physics of Lipids*, 4(2), pp.109–130. Available at: http://linkinghub.elsevier.com/retrieve/pii/0009308470900423 [Accessed July 24, 2017].

Sheiner, L.B. & Beal, S.L., 1981. Some suggestions for measuring predictive performance. *Journal of pharmacokinetics and biopharmaceutics*, 9(4), pp.503–12. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7310648 [Accessed July 20, 2018].

Shugarts, S. & Benet, L.Z., 2009. The role of transporters in the pharmacokinetics of orally administered drugs. *Pharmaceutical research*, 26(9), pp.2039–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19568696 [Accessed August 17, 2018].

Sim, D.S.M., 2015. Drug Absorption and Bioavailability. In *Pharmacological Basis of Acute Care*. Cham: Springer International Publishing, pp. 17–26. Available at: http://link.springer.com/10.1007/978-3-319-10386-0_3 [Accessed June 26, 2018].

Singh, B.N., 1999. Effects of Food on Clinical Pharmacokinetics. *Clinical Pharmacokinetics*, 37(3), pp.213–255. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10511919 [Accessed August 15, 2018].

Söderlind, E. et al., 2010. Simulating fasted human intestinal fluids: understanding the roles of lecithin and bile acids. *Molecular pharmaceutics*, 7(5), pp.1498–507. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20698569 [Accessed December 10, 2015].

Solvang, S. & Finholt, P., 1970. Effect of Tablet Processing and Formulation Factors on Dissolution Rate of the Active Ingredient in Human Gastric Juice. *Journal of Pharmaceutical Sciences*, 59(1), pp.49–52. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022354915371641 [Accessed July 21, 2017].

Stappaerts, J. et al., 2014. Human and simulated intestinal fluids as solvent systems to explore food effects on intestinal solubility and permeability. *European Journal of*

Pharmaceutical Sciences, 63, pp.178–186.

Steingoetter, A. et al., 2006. Effects of posture on the physiology of gastric emptying: A magnetic resonance imaging study. *Scandinavian Journal of Gastroenterology*, 41(10), pp.1155–1164. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16990200 [Accessed August 12, 2018].

Storer, B.E., 1989. Design and analysis of phase I clinical trials. *Biometrics*, 45(3), pp.925–37. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2790129 [Accessed August 22, 2018].

Stotzer, P.O. & Abrahamsson, H., 2000. Human postprandial gastric emptying of indigestible solids can occur unrelated to antral phase III. *Neurogastroenterology and Motility*, 12(5), pp.415–419. Available at: http://doi.wiley.com/10.1046/j.1365-2982.2000.00218.x [Accessed December 12, 2015].

Stuart, M. & Box, K., 2005. Chasing equilibrium: measuring the intrinsic solubility of weak acids and bases. *Analytical chemistry*, 77(4), pp.983–90. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15858976 [Accessed July 6, 2017].

Sugano, K., 2009. Computational oral absorption simulation for low-solubility compounds. *Chemistry and Biodiversity*, 6(11), pp.2014–2029. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19937838 [Accessed April 21, 2017].

Sun, D. et al., 2004. In vitro testing of drug absorption for drug “developability” assessment: forming an interface between in vitro preclinical data and clinical outcome. *Current opinion in drug discovery & development*, 7(1), pp.75–85. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14982151 [Accessed September 5, 2018].

Sunesen, V.H., Vedelsdal, R., et al., 2005. Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug. *European Journal of Pharmaceutical Sciences*, 24(4), pp.297–303.

Sunesen, V.H., Pedersen, B.L., et al., 2005. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 24(4), pp.305–13. Available at: http://www.sciencedirect.com/science/article/pii/S0928098704002945 [Accessed December 14, 2015].

Tanaka, Y. et al., 2015. Impact of Luminal Fluid Volume on the Drug Absorption After Oral

Administration: Analysis Based on In Vivo Drug Concentration–Time Profile in the Gastrointestinal Tract. *Journal of Pharmaceutical Sciences*, 104(9), pp.3120–3127. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022354916300910 [Accessed August 19, 2018].

Tanaka, Y. et al., 2012. Nanoparticulation of probucol, a poorly water-soluble drug, using a novel wet-milling process to improve *in vitro* dissolution and *in vivo* oral absorption. *Drug Development and Industrial Pharmacy*, 38(8), pp.1015–1023. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22118063 [Accessed August 8, 2018].

Thelen, K. et al., 2011. Evolution of a detailed physiological model to simulate the gastrointestinal transit and absorption process in humans, part 1: oral solutions. *Journal of pharmaceutical sciences*, 100(12), pp.5324–45. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21993815 [Accessed December 17, 2015].

Thelen, K. et al., 2012. Evolution of a Detailed Physiological Model to Simulate the Gastrointestinal Transit and Absorption Process in Humans, Part II: Extension to Describe Performance of Solid Dosage Forms. *Journal of Pharmaceutical Sciences*, 101(3), pp.1267–1280. Available at: http://linkinghub.elsevier.com/retrieve/pii/S0022354915316798 [Accessed February 12, 2018].

Tistaert, C. et al., 2018. Food Effect Projections via Physiologically Based Pharmacokinetic Modeling: Predictive Case Studies. *Journal of Pharmaceutical Sciences*, pp.1–11. Available at: https://doi.org/10.1016/j.xphs.2018.05.024 [Accessed September 4, 2018].

Le Tourneau, C., Lee, J.J. & Siu, L.L., 2009. Dose escalation methods in phase i cancer clinical trials. *Journal of the National Cancer Institute*, 101(10), pp.708–720. Available at: http://www.ncbi.nlm.nih.gov/pubmed/19436029 [Accessed August 22, 2018].

Upton, R.N., Foster, D.J.R. & Abuhelwa, A.Y., 2016. An introduction to physiologically-based pharmacokinetic models. *Paediatric Anaesthesia*, 26(11), pp.1036–1046.

Veering, B.T. et al., 1990. The effect of age on serum concentrations of albumin and alpha 1-acid glycoprotein. *British journal of clinical pharmacology*, 29(2), pp.201–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2306411 [Accessed August 23, 2018].

Vertzoni, M. et al., 2004. Dissolution media simulating the intralumenal composition of the small intestine: physiological issues and practical aspects. *The Journal of pharmacy and pharmacology*, 56(4), pp.453–62. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15099440 [Accessed December 14, 2015].

Vertzoni, M. et al., 2007. Estimation of intragastric solubility of drugs: in what medium? *Pharmaceutical research*, 24(5), pp.909–17. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17372688 [Accessed December 10, 2015].

Vertzoni, M. et al., 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. *European Journal of Pharmaceutics and Biopharmaceutics*, 60(3), pp.413–417. Available at: http://www.sciencedirect.com/science/article/pii/S0939641105001062 [Accessed October 19, 2015].

Volpe, D.A., 2010. Application of method suitability for drug permeability classification. *The AAPS journal*, 12(4), pp.670–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20811966 [Accessed July 7, 2017].

Volpe, D.A., 2008. Variability in Caco-2 and MDCK Cell-Based Intestinal Permeability Assays. *Journal of Pharmaceutical Sciences*, 97(2), pp.712–725. Available at: https://www.sciencedirect.com/science/article/pii/S0022354916324996 [Accessed August 17, 2018].

Wagner, C. et al., 2012. Predicting the oral absorption of a poorly soluble, poorly permeable weak base using biorelevant dissolution and transfer model tests coupled with a physiologically based pharmacokinetic model. *European Journal of Pharmaceutics and Biopharmaceutics*, 82(1), pp.127–138. Available at: https://www.sciencedirect.com/science/article/pii/S0939641112001750#f0015 [Accessed August 24, 2018].

Wagner, C. et al., 2013. Utilizing In Vitro and PBPK Tools to Link ADME Characteristics to Plasma Profiles: Case Example Nifedipine Immediate Release Formulation. *Journal of Pharmaceutical Sciences*, 102, pp.3205–3219. Available at: https://ac.els-cdn.com/S0022354915309540/1-s2.0-S0022354915309540-main.pdf?_tid=ebfcfa44-0511-11e8-b854-00000aacb35f&acdnat=1517243697_970f27c36ac33bb54732a025645f9699 [Accessed January 29, 2018].

Walle, T. et al., 2004. High absorption but very low bioavailability of oral resveratrol in humans. *Drug metabolism and disposition: the biological fate of chemicals*, 32(12), pp.1377–82. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8565785 [Accessed August 17, 2018].

Wasserman, E. et al., 1997. Severe CPT-11 toxicity in patients with Gilbert's syndrome: two

case reports. *Annals of oncology : official journal of the European Society for Medical Oncology*, 8(10), pp.1049–51. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9402181 [Accessed July 13, 2018].

Welling, P.G., 1996. Effects of Food on Drug Absorption. *Annual Review of Nutrition*, 16(1), pp.383–415. Available at: http://www.annualreviews.org/doi/10.1146/annurev.nu.16.070196.002123.

Welling, P.G., 1977. Influence of food and diet on gastrointestinal drug absorption: A review. *Journal of Pharmacokinetics and Biopharmaceutics*, 5(4), pp.291–334. Available at: http://link.springer.com/10.1007/BF01061694 [Accessed August 18, 2018].

Wiedmann, T.S., Liang, W. & Kamel, L., 2002. Solubilization of drugs by physiological mixtures of bile salts. *Pharmaceutical research*, 19(8), pp.1203–8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12240947 [Accessed July 20, 2016].

Willmann, S. et al., 2003. PK-Sim®: a physiologically based pharmacokinetic 'whole-body' model. *BIOSILICO*, 1(4), pp.121–124. Available at: https://www.sciencedirect.com/science/article/pii/S1478538203023424?via%3Dihub [Accessed July 11, 2018].

Willmann, S., Lippert, J. & Schmitt, W., 2005. From physicochemistry to absorption and distribution: predictive mechanistic modelling and computational tools. *Expert Opinion on Drug Metabolism & Toxicology*, 1(1), pp.159–168. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16922658 [Accessed April 9, 2019].

Woo, J. et al., 1994. Effect of age and disease on two drug binding proteins: albumin and alpha-1- acid glycoprotein. *Clinical biochemistry*, 27(4), pp.289–92. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8001290 [Accessed August 23, 2018].

Wu, G., 1995. Calculating predictive performance: A user's note. *Pharmacological Research*, 31(6), pp.393–399. Available at: https://www.sciencedirect.com/science/article/pii/1043661895800956 [Accessed July 20, 2018].

Yalkowsky, S.H. & Valvani, S.C., 1980. Solubility and partitioning I: Solubility of nonelectrolytes in water. *Journal of pharmaceutical sciences*, 69(8), pp.912–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7400936 [Accessed July 26, 2018].

Yeo, K.R., Jamei, M. & Rostami-Hodjegan, A., 2013. Predicting drug–drug interactions: application of physiologically based pharmacokinetic models under a systems biology

approach. *Expert Review of Clinical Pharmacology*, 6(2), pp.143–157. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23473592 [Accessed July 13, 2018].

Yu, L.X. et al., 2002. Biopharmaceutics classification system: the scientific basis for biowaiver extensions. *Pharmaceutical research*, 19(7), pp.921–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12180542 [Accessed August 17, 2018].

Yu, L.X. et al., 1996. Transport approaches to the biopharmaceutical design of oral drug delivery systems: prediction of intestinal absorption. *Advanced drug delivery reviews*, 19(3), pp.359–76. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11540095 [Accessed July 13, 2018].

Zaitseva, T.M. et al., 1995. Pharmacokinetics of probucol dosage forms in clinical tests. *Pharmaceutical Chemistry Journal*, 29(4), pp.245–247. Available at: http://link.springer.com/10.1007/BF02219544 [Accessed August 8, 2018].

Zhang, Y. et al., 2010. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine*, 99(3), pp.306–314. Available at: http://dx.doi.org/10.1016/j.cmpb.2010.01.007.

Zhou, Z. et al., 2017a. Influence of Physiological Gastrointestinal Surfactant Ratio on the Equilibrium Solubility of BCS Class II Drugs Investigated Using a Four Component Mixture Design. *Molecular Pharmaceutics*, p.acs.molpharmaceut.7b00354. Available at: http://pubs.acs.org/doi/abs/10.1021/acs.molpharmaceut.7b00354.

Zhou, Z. et al., 2017b. Statistical investigation of simulated fed intestinal media composition on the equilibrium solubility of oral drugs. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, 99, pp.95–104. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27940083 [Accessed August 1, 2018].

Zhuang, X. & Lu, C., 2016. PBPK modeling and simulation in drug research and development. *Acta Pharmaceutica Sinica B*, 6(5), pp.430–440. Available at: http://dx.doi.org/10.1016/j.apsb.2016.04.004.

8. Appendix

Appendix I Published scientific article

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Statistical investigation of the full concentration range of fasted and fed simulated intestinal fluid on the equilibrium solubility of oral drugs

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ABSTRACT

Upon oral administration the solubility of a drug in intestinal fluid is a key property influencing bioavailability. It is also recognised that simple aqueous solubility does not reflect intestinal solubility and to optimise in vitro investigations simulated intestinal media systems have been developed. Simulated intestinal media which can mimic either the fasted or fed state consists of multiple components each of which either singly or in combination may influence drug solubility, a property that can be investigated by a statistical design of experiment technique. In this study a design of experiment covering the full range from the lower limit of fasted to the upper limit of fed parameters and using a small number of experiments has been performed. The measured equilibrium solubility values are comparable with literature values for simulated fasted and fed intestinal fluids as well as human fasted and fed intestinal fluids. The equilibrium solubility data range is statistically equivalent to a combination of published fasted and fed design of experiment data in six (indomethacin, phenytoin, zafirlukast, carvedilol, fenofibrate and probucol) drugs with three (aprepitant, tadalafil and felodipine) drugs not equivalent. In addition the measured equilibrium solubility data sets were not normally distributed. Further studies will be required to determine the reasons for these results however it implies that a single solubility measurement without knowledge of the solubility distribution will be of limited value. The statistically significant media factors which promote equilibrium solubility (pH, sodium oleate and bile salt) were in agreement with published results but the number of determined significant factors and factor interactions was fewer in this study, lecithin for example did not influence solubility. This may be due to the reduction in statistical sensitivity from the lower number of experimental data points or the fact that using the full range will examine media parameters ratios that are not biorelevant. Overall the approach will provide an estimate of the solubility range and the most important media factors but will not be equivalent to larger scale focussed studies. Further investigations will be required to determine why some drugs do not produce equivalent DoE solubility distributions, for example combined fasted and fed DoE, but this simply may be due to the complexity and individuality of the interactions between a drug and the media components.

Appendix II Poster presented at the Pharmacokinetic United Kingdom (PKUK) Conference in London in November 2016

Statistical investigation of the composition of simulated intestinal fluid on the equilibrium solubility of oral drugs.

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Background: Simulated gastrointestinal media of the fasted and fed states in human have been developed to assist in vitro studies but the interactions between all of its constituents remain complex. A statistical design of experiment (DoE) approach was previously used to assess these interactions. This approach was employed to investigate the influence of simulated gastrointestinal media composition in the fasted and fed state on the equilibrium solubility of twelve BCS class II compounds (four acids, four bases and four neutrals). The solubility range was higher in the fed state and overall pH and concentration of sodium oleate, bile salt and lecithin showed the most significant influence on solubility.
Aim: To determine the feasibility of combining a fasted and fed study into one "combined design of experiment" in order to obtain comparable results regarding the solubility range and the influence of gastrointestinal media components.
Method: A quarter of the full factorial design of experiment with 7 factors (bile salt, lecithin, sodium oleate, pH, salt, buffer and monoglyceride) and 2 levels (upper and lower limits) was constructed using Minitab®17.2.1. Minitab generated 32 different experiments by various combinations of the different limits. Nine BCS class II compounds were tested (two acids, three neutrals and four bases). A weight of powdered drug (10mg) was added to 15 ml centrifuge tubes. The amount of stock solution and water was added to provide a final volume of 4 ml. pH was then adjusted to 5 or 7. Tubes were shaken for 1 hour at room temperature and then pH adjusted again as before. Tubes are then placed in an orbital shaker and incubated for 24 hr at 37 °C and 240 rpm. Following incubation the tubes were centrifuged. The supernatant was removed to determine the solubilised drug concentration by HPLC. Minitab was then used to calculate individual and interactions factor's standardised effect on the measured solubility.
Results: The measured equilibrium solubility values indicate that the combined DoE covered the solubility space of the previous fasted and fed DoE for most of the compounds. The components showing the lowest effect on the solubility are buffer, salt, monoglyceride and lecithin (0 significant from 9 drugs) followed by sodium oleate (2 significant). The media with components with the biggest influence are bile salt (4 significant) and pH (6 significant). This is consistent with the fed state experiment where pH and bile salt were the most significant (12 and 10 significant from 13 respectively) but contrasting with the fasted experiment where pH and sodium oleate were the most influential (10 significant from 12). Among all the combinations of factors with each drug a statistical significance is only present five times from the hundreds of possibilities. Three interactions were significant for zafirlukast (Bile salt* sodium oleate, bile salt*pH and lecithin*buffer) and two for indomethacin (Bile salt*pH and lecithin*buffer). In the previous fasted study only one third of all the possible interactions were significant while in the fed study neutral drugs displayed a more complicated picture with fifteen of the possible twenty six interactions significant.

Conclusion: The experiment provided comparable solubility results to the previous fasted and fed studies. This suggests that a combined DoE could be sufficient to explore the intestinal solubility in biorelevant media. Overall pH, bile salt and sodium oleate were dominant although they were not significant for all compounds. However the statistical outcome highlights the complexity of assessing the interactions between the intestinal components.

Appendix III Poster presented at the Advanced Process Modelling (APM) Forum in London in April 2017

Statistical investigation of the composition of simulated intestinal fluid on the equilibrium solubility of oral drugs and *in silico* prediction using gCOAS.

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Objective: A statistical design of experiment (DoE) approach was employed to investigate the influence of simulated intestinal fluids (SIF) composition on the equilibrium solubility of BCS class II compounds. The aim is to enhance understanding of how orally-administered drugs are taken up from the intestinal tract and combine this knowledge with *in silico* models to predict in the early stages of development the absorption and therefore the performance of these compounds.

Method: Using Minitab® 17.2.1 a DoE was constructed in the fasted and in the fed state with 7 and 8 factors respectively and 2 levels (upper and lower limits of factors). Thirteen BCS class II compounds were tested (acids, bases and neutrals). The PSE software gCOAS 1.3.0 was then used to predict the solubility of nine compounds (two acids, four bases and two neutrals) and compared to the experimental data previously generated.

Results: The fasted state experiment proved the feasibility of this systematic approach, simulated the inherent solubility variabilities and determined the key factors controlling solubility. The fed state experiment confirmed the suitability of this approach expanded to the food effect with logically higher solubility values. Similar results were observed for acidic and basic drugs while neutral drugs behaved differently. The simulations were challenging for the correlation of simulated vs experimental solubility values. The results showed a compound specificity however a refinement of the simulations would certainly improve the correlation.