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![UCC crest] **University College Cork, Ireland**
Coláiste na hOllscoile Corcaigh

Mechanistic understanding of bioenabling formulation approaches to improve oral bioavailability using porcine *in vivo* and *in silico* models

Thesis Presented by

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In fulfilment of the requirements for the degree of

Doctor of Philosophy

under the supervision of

Dr Brendan T. Griffin, *B.Sc. (Pharm.), Ph.D., M.P.S.I.*

and

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Head of School;

Prof Stephen Byrne

August, 2018

Contents

Declaration

This thesis has not been previously submitted, in part or in whole, to this or any other university for any degree and is, except where duly noted and acknowledged, the original work of the author.

Author Contribution

All work was performed independently by the author, with the following exceptions:

Chapter 2

Dr Kalpa Nagarsekar carried out formulation preparation and biorelevant dissolution testing. Dr Brendan Griffin and Mr Vincent Mehigan assisted during the oral bioavailability study in pigs.

Chapter 3

Dr Waleed Faisal carried out physiochemical analysis. Dr Waleed Faisal and Dr Brendan Griffin performed the oral bioavailability study in pigs. Dr Waleed Faisal analysed plasma concentrations of fenofibric acid.

Chapter 4

Dr Brendan Griffin and Mr Vincent Mehigan assisted during the oral bioavailability study in pigs.

Chapter 5

Dr Brendan Griffin, Mr Vincent Mehigan, Mr Niklas Köhl and Ms Laura Henze assisted during the oral bioavailability study. Ms Laura Henze assisted during analysis of plasma samples and characterisation of gastrointestinal fluid.

Signed: _____   Date: _____

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"No one can whistle a symphony; it takes an orchestra to play it."

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iv

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v

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Go raibh míle maith agaibh go léir agus rath Dé oraibh.

Author Publications

Peer reviewed publications associated with this thesis

1. **Chapter three** - Lipidic dispersion to reduce food dependent oral bioavailability of fenofibrate: *in vitro*, *in vivo* and *in silico* assessments

   Joseph P. O'Shea, Waleed Faisal, Therese Ruane-O'Hora, Ken J. Devine, Caitriona M. O'Driscoll, Brendan T. Griffin

   European Journal of Pharmaceutics and Biopharmaceutics, Volume 96, October 2016, pages 207-216

2. **Chapter two** - Mesoporous silica-based dosage forms improve bioavailability of poorly soluble drugs in pigs: case example fenofibrate

   Joseph P O'Shea, Kalpa Nagarsekar, Alena Wieber, Vanessa Witt, Elisabeth Herbert, Caitriona M O'Driscoll, Christoph Saal, Dieter Lubda, Brendan T Griffin, Jennifer B Dressman

   Journal of Pharmacy and Pharmacology, Volume 69, Issue 10, October 2017, Pages 1284-1292

3. **Chapters one and six –** The pig as a pre-clinical model for predicting oral bioavailability and *in vivo* performance of pharmaceutical oral dosage forms - a PEARRL review

   Laura J. Henze, Niklas J. Koehl, **Joseph P. O'Shea**, Edmund Kostewicz, René Holm, Brendan T. Griffin

   Journal of Pharmacy and Pharmacology, 2018, *In Press, doi: 10.1111/jphp.12912*

4. **Chapter one –** Lipid Based Formulations

   In *Encyclopaedia of Controlled Drug Delivery, Second Edition, In Press*

   Joseph P. O'Shea, Caitriona M. O'Driscoll, Brendan T. Griffin

5. **Chapters one and six –** Food for thought: Formulating away the food effect - a PEARRL review

    Joseph P. O'Shea, René Holm, Caitriona M. O'Driscoll, Brendan T. Griffin

    Journal of Pharmacy and Pharmacology, 2018, *In Press, doi: 10.1111/jphp.12957*

Conference presentations

1. **Joseph P. O'Shea**, Waleed Faisal, Ken J. Devine Caitriona M. O'Driscoll, Brendan T. Griffin. *A novel lipidic dispersion to reduce the food effect on bioavailability of fenofibrate: in vitro, in vivo and in silico assessments*.
    Poster presented at UKICRS symposium, UCC, Cork, 10th – 11th April 2014.

2. **Joseph P. O'Shea**, Waleed Faisal, Ken J. Devine Caitriona M. O'Driscoll, Brendan T. Griffin. *A novel lipidic dispersion to reduce the food effect on bioavailability of fenofibrate: in vitro, in vivo and in silico assessments*.
    Poster presented at All Ireland School of Pharmacy Research Seminar, Trinity College, Dublin, 14th – 15th April 2014.

3. **Joseph P. O´Shea**, Gerlinde F. Born, Caitriona M. O'Driscoll, Brendan T. Griffin, Jennifer B. Dressman.
    Dissolution and in vivo performance of a commercial celecoxib formulation.
    Poster presented at AAPS Annual Meeting and Exposition, Orange County Convention Centre, Orlando, Florida, USA, 25th-29th October 2015

4. **Joseph P. O´Shea**, Alena Wieber, Christoph Saal, Brendan T. Griffin, Vanessa Witt, Kalpa Nagarsekar, Elisabeth Herbert, Jennifer B. Dressman, Dieter Lubda.
    Mesoporous Silica for Improving Oral Bioavailability of Fenofibrate: In Vivo Evaluation
    Poster presented at AAPS Annual Meeting and Exposition, Colorado Convention Centre, Denver, Colorado, USA, 13th – 17th November 2016

5. **Joseph P. O'Shea**, Kalpa Nagarsekar, Alena Wieber, Vanessa Witt, Elisabeth Herbert, Christoph Saal, Dieter Lubda, Caitriona M. O'Driscoll, Jennifer B. Dressman, Brendan T. Griffin.

   Mesoporous silica based dosage forms improve bioavailability of poorly soluble drugs in pigs: case example fenofibrate.

   Poster presented at All Ireland School of Pharmacy Research Seminar, University College Cork, 24th – 25th April 2017.

6. **Joseph P. O'Shea.**

   Strategies to aid early screening and translation of bioenabling formulations – a focus on lipid based formulations.

   Oral presentation at European Drug Absorption Network 15th Meeting, Katholieke Universiteit Leuven, Leuven, Belgium, 19th – 21st March 2017

Concomitant Peer Reviewed Publications

1. Folate-targeted amphiphilic cyclodextrin.siRNA nanoparticles for prostate cancer therapy exhibit PSMA mediated uptake, therapeutic gene silencing *in vitro* and prolonged circulation *in vivo*

   James C. Evans, Meenakshi Malhotra, Jianfeng Guo, **Joseph P. O'Shea**, Karen Hanrahan, Amanda O'Neill, William D. Landry, Brendan T. Griffin, Raphael Darcy, R. William Watson, Caitriona M. O'Driscoll

   Nanomedicine: Nanotechnology, Biology and Medicine, Volume 12, Issue 8, November 2016, pages 2341-2351

2. *In vitro* dissolution models for the prediction of *in vivo* performance of an oral mesoporous silica formulation

   Carol A. McCarthy, Waleed Faisal, **Joseph P. O'Shea**, Colm Murphy, Robert J. Ahern, Katie B. Ryan, Brendan T. Griffin, Abina M. Crean

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Abstract

With the ever-increasing prevalence of poorly soluble compounds in drug development pipelines, the identification of compounds with poor 'developability' owing to sub-optimal absorption properties has led to formulation and delivery challenges in drug development. There is a consequent need to develop both novel formulations that overcome the solubility limitations of poorly water soluble drugs (PWSD), along with a range of predictive *in vitro* and *in silico* biopharmaceutics based tools for guiding formulation design and forecasting *in vivo* performance. This thesis aims to assess both novel bioenabling formulations and new *in vitro* and *in silico* tools to predict their *in vivo* performance in pigs as a means to improve efficiency in formulation development.

This thesis has, firstly, demonstrated the ability of two novel bioenabling approaches to improve oral bioavailability of fenofibrate in fasted pigs. Secondly, the utility of *in vitro* and *in silico* tools to predict *in vivo* performance in fasted pigs has been investigated. Thirdly, the ability of the pig to act as a model of human bioavailability, as well as its suitability to act as an *in vivo* screening tool for bioenabling approaches for PWSD has been described. Finally, limitations of the pig model for assessment of food effect using current approaches have been identified, and suggestions for future characterisation have been made. Overall, the utility of the pig in assessing bioenabling approaches has been demonstrated. Concomitantly, the predictive ability of *in vitro* and *in silico* biopharmaceutical tools has been demonstrated. Thus, the pre-clinical pig model has proven useful in the assessment of both bioenabling formulations and the predictive capacity of biorelevant biopharmaceutical tools.

Abbreviations

% w/v	Percentage weight per volume
% w/w	Percentage weight per weight
°C	Degrees Celsius
ABL	Aqueous boundary layer
ADMET	Absorption, distribution, metabolism, excretion and toxicology
AEEC	Animal Experimentation Ethics Committee
Å	Angstrom
ANOVA	Analysis of variance
ASD	Amorphous solid dispersion
AUC	Area under the curve
BCRP	Breast cancer resistance protein
BCS	Biopharmaceutical classification system
BDDCS	Biopharmaceutical drug disposition classification system
C_{eq}	Equilibrium solubility
C_{max}	Peak drug plasma concentration
CoV	Coefficient of Variation
COX-2	Cylooxygenase-2
CQA	Critical quality attributes
CYP450	Cytochromes P450
DCS	Developability classification system
DE/ %DE	Dissolution efficiency
DG	Diglyceride
DGM	Dynamic gastric model
D/P	Dissolution/permeation
DSC	Differential scanning calorimetry
EM	Extensive metaboliser
EMA	European Medicines Agency
EU	European Union
F	Bioavailability
F_2	Similarity factor
F_a	Fraction absorbed
FA	Fatty acid
FaSSGF	Fasted state simulated gastric fluid
FaSSIF	Fasted state simulated intestinal fluid
FaSSIF-V2	Fasted state simulated intestinal fluid-version two
FD	Fold difference
FDA	Food and Drug Administration
FeSSIF	Fasted state simulated intestinal fluid

FeSSIF-V2	Fasted state simulated intestinal fluid-version two
FF-SLC	Fenofibrate loaded mesoporous silica
FPM	First pass metabolism
g	Times gravity
g	Grams
GI	Gastrointestinal
GIT	Gastrointestinal tract
HFF	High fat fed
HPLC	High performance liquid chromatography
HPLC-UV	High performance liquid chromatography – ultraviolet detection
HPMC	Hydroxypropyl methylcellulose
HPMCAS	Hydroxypropyl methylcellulose acetate succinate
hr	Hours
IR	Immediate release
i.v.	Intravenous
IVIVC	*In vitro- in vivo* correlation
IVIVR	*In vitro- in vivo* relationship
kg	Kilograms
kV	Kilovolt
L	Litres
λ	Wavelength
LBF	Lipid based formulation
LCFA	Long chain fatty acid
LCT	Long chain triglyceride
LD	Lipidic dispersion
LFF	Low fat fed
LOD	Limit of detection
LogD	Logarithmic distribution coefficient
LogP	Logarithmic partition coefficient
LOQ	Limit of quantification
M	Molar
mA	Milliamps
MCT	Medium chain triglyceride
MFF	Medium fat fed
mg	Milligrams
MG	Monoglyceride
min	Minutes
ml	Millilitres
mm	Millimetres
MRP	Multidrug resistance protein

Mw	Molecular weight
ng	nanograms
nm	nanometres
NSAID	Non-steroidal anti-inflammatory drug
OATP	Organic anion transporter peptide
P$_{app}$	Apparent human permeability
PBPK	Physiologically based pharmacokinetic model
PEG	Polyethylene glycol
PES	Polyether sulfone
P-gp	Permeability glycoprotein transporter
PK	Pharmacokinetics
pKa	logarithmic acid dissociation constant
PM	Poor metaboliser
PPI	Polymeric precipitation inhibitor
PVP	Polyvinylpyrrolidone/ povidone
PVPVA	Polyvinylpyrrolidone vinyl acetate**/ Copovidone**
PWSD	Poorly water soluble drug
PXRD	Powder X-ray diffraction
QbD	Quality by design
R&D	Research and development
RC-GF	Regenerated cellulose- glass fibre
Ro5	Rule of five (Lipinski's)
rpm	Revolutions per minute
SD	Standard deviation
SE/SEM	Standard error of the mean
Sec	Seconds
SEDDS	Self-emulsifying drug delivery systems
SE$_{FD}$	Standard error of the fold difference
SIF	Simulated intestinal fluid
SLC	Mesoporous silica
TG	Triglyceride
TJ	Tight junction
T$_{lag}$	Lag time
T$_{max}$	Time at which C$_{max}$ is observed
USP	United states pharmacopoeia
UV-vis	Ultraviolet visible spectroscopy
ver.	Version
μg	Micrograms
μL	Microlitres
μm	Micrometres

Chapter 1 : Introduction

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Lipid Based Formulations

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Joseph P. O'Shea, Caitriona M. O'Driscoll, Brendan T. Griffin

And

The pig as a pre-clinical model for predicting oral bioavailability and *in vivo* performance of pharmaceutical oral dosage forms - a PEARRL review

Laura J. Henze, Niklas J. Koehl, Joseph P. O'Shea, Edmund Kostewicz, René Holm, Brendan T. Griffin

Journal of Pharmacy and Pharmacology, 2018, *In Press, doi: 10.1111/jphp.12912*

And

Food for thought: Formulating away the food effect - a PEARRL review

Joseph P. O'Shea, René Holm, Caitriona M. O'Driscoll, Brendan T. Griffin

Journal of Pharmacy and Pharmacology, *Under Review*

Strategies to improve 'developability' in drug product R&D

A significant focus for drug development scientists over the last number of decades has been the 'developability' of new chemical entities and current drug candidates. In that time there has been a significant shift towards discovery of candidate drugs that display less than optimal drug like properties, particularly with regard to solubility and/or lipophilicity (Butler and Dressman, 2010). This trend has variously been ascribed to the biology of the drug target, the methods of drug screening and discovery and nature of combinatorial chemistry utilised in drug development (Bergström et al., 2016). The advent of modern drug discovery techniques, such as combinatorial chemistry and high throughput screening, has led to a distinct trend towards lead drug candidates with optimized pharmacodynamic properties, but poor 'developability' owing to sub-optimal absorption properties. Developability, in this context, is widely used to describe just how 'drug like' a molecule is, with regard to its physicochemical and biopharmaceutical characteristics, with a particular emphasis on the absorption, distribution, metabolism, excretion and toxicology (ADMET) process (Saxena et al., 2009). The identification of such limitations in drug molecules has led to increased candidate attrition in development pipelines.

The need to establish a framework to diagnose these biopharmaceutical limitations of new drug candidates led to the establishment of numerous classification systems, most notably the biopharmaceutical classification system (BCS), which classified compounds based on their solubility and permeability (Amidon et al., 1995), the biopharmaceutical drug disposition classification system (BDDCS) (Wu and Benet, 2005), the rule of five (Ro5) (Lipinski et al., 1997) and the developability classification

system (DCS) (Butler and Dressman, 2010). However, despite major advancements in understanding the key factors influencing absorption there is actually evidence that the number of drugs in development pipelines displaying these limitations has increased and it is estimated that anywhere from 40%-70% of current drug candidates display poor solubility, such that their absorption is compromised (Bergström et al., 2016). These molecules display solubility or dissolution rate limited bioavailability, where complete dissolution would take longer than the transit time past the absorptive region of the gastrointestinal tract (GIT), or require a larger volume of fluid than that which is present in the GIT (Butler and Dressman, 2010). These factors contribute towards limiting oral bioavailability, and the formulation and delivery of these poorly water soluble drugs (PWSD) poses a major challenge to their successful development into new medicines, where delays in development or even failure to gain approval can occur. Increasing lipophilicity associated with these poorly soluble drugs also leads to increased susceptibility to food mediated alterations in bioavailability, particularly an increased likelihood of a positive food effect (Custodio et al., 2008, Raman and Polli, 2016). Such food effects are considered as limiting factors in drug development, where there are clinical and commercial preferences for drug/ formulation combinations which are resistant to food mediated changes in bioavailability. Consequently, a significant body of research has focused on formulating these compounds to overcome food effects (Lentz, 2008, Schmidt and Dalhoff, 2002). An alternative approach is the use of label claims that a medicine should be taken in a specific prandial state, though this approach can result in low levels of adherence in certain patient populations, limiting clinical utility (Todd et al., 2012, Singh and Malhotra, 2004, Kang and Ratain, 2010, Thombre et al., 2011).

3

Successful delivery of these challenging compounds often relies on the use of novel bioenabling formulations, designed to enhance their *in vivo* solubility and/or dissolution. In this regard, novel formulation approaches, such as nanoformulations, solid dispersions and lipid based formulations are increasingly being used in drug development pipelines, however, there are still gaps in the knowledge of formulation of PWSD and technologies to increase exposure (Kwong, 2015, Lennernas et al., 2014). A key reluctance in the industry appears a perceived risk associated with the early stage selection of bioenabling formulations – reflecting a lack of guidance on how to select an appropriate bioenabling formulation. As a result, working in restricted drug development timelines, pharmaceutical development scientists have been known to formulate drugs using conventional formulation approaches in pre-clinical testing, yielding sup-optimal bioavailability and wasted resources. Developing an understanding of the key biopharmaceutical properties and how they affect the ADMET process can help to drive efficiencies in the formulation screening process, which can otherwise be a predominantly iterative process that may still result in failure to achieve adequate exposure (Kwong, 2015, Bergstrom et al., 2014). Introducing more clinically relevant screening methods, especially in the context of quality by design (QbD), could make development more cost-effective while maintaining quality (Lennernas et al., 2014). Development of predictive biopharmaceutical tools and the validation of existing approaches with respect to their performance in predicting the *in vivo* outcomes is central to this process. This is one of the assortment of limitations which has led to declining productivity in pharmaceutical research and development (R&D) productivity over the last six decades.

4

Declining productivity in Pharmaceutical drug product development

Over the last few decades, despite huge advances in therapeutic target discovery, the number of new medicines approved for clinical use has decreased consistently. For example, the number of new medicines approved per $1 billion invested in R&D has decreased by 50% every nine years since the 1950s, reflecting the high attrition rates encountered in translating drug molecules to medicines (Scannell et al., 2012). Numerous industry reports have indicated there is a clear need to develop new technologies to improve the developability of emerging drug candidates and to unlock key bottle-necks stifling innovation in pharmaceutical development (Arrowsmith, 2011). With upwards of 90% of drug candidates failing to ever gain approval, and with approximately 30-40% phase III clinical trials failing, there is significant financial and time costs associated with failures in drug development pipelines (Hay et al., 2014). Consideration must be given to the impact of these high failure rates on the cost of drug development and how best to incentivise continued focus on breakthrough medicines (Calcoen et al., 2015). Poor biopharmaceutic and pharmacokinetic (PK) properties are among the direct causes of this attrition, and such drug candidates can consequently take more time and resources to develop, and may make efficacy failures more likely (Kostewicz et al., 2014b, Hann and Keseru, 2012).

In order to overcome these challenges, a major focus of R&D in pharmaceutical industry is reduction of costs and development cycle time-frames. Through utilising earlier proof of concept studies and focusing on the 'R&D sweet-spot' prior to phase II clinical trials a 'quick win, fast fail' can be achieved, improving phase II success rates

(Paul et al., 2010). Attrition is the single biggest determinant of R&D efficiency, and reducing attrition, particularly at stage II and stage III, will have profound effects on the cost of developing new medicines (Paul et al., 2010). To achieve this, a major goal is to shift compound and formulation attrition earlier in the development process, with key "go/no-go" decisions occurring even prior to the clinical stage (Paul et al., 2010, Kwong, 2015).

Any strategy to streamline formulation development to meet the accelerated time-lines, therefore, needs to tackle both R&D inefficiencies and reduce R&D costs and delays, through improving the predictivity of early screening and avoiding unnecessary testing. Considering the limitations of current R&D approaches outlined above, and recognizing that most new drugs are poorly soluble, the drive to accelerate the development and approval of break-through therapy drugs urgently requires an accelerated development paradigm, consisting of three key elements:

1. Deign of innovative, bioenabling formulations for poorly soluble drugs, with choice guided through knowledge harnessed in formulation screening

2. Generation of predictive *in vitro* and *in silico* tools capable of rapidly and accurately screening formulations and predicting their ability to deliver drug *in vivo*

3. Improving the predictive capacity of pre-clinical *in vivo* testing by prudent choice of animal model and understanding of the key biopharmaceutical properties of model choice

6

The current thesis focuses on these key questions, through the pre-clinical *in vitro*, *in vivo* and *in silico* assessment of two novel bioenabling formulations and the absorption enhancing capacity of co-administration of PWSD with food in the pig model, with a specific focus on eliminating the food effect. The dual aim is to assess the capability of these bioenabling approaches to enhance the bioavailability of PWSD, while also examining the predictive capacity of the range of biorelevant *in vitro*, *in silico* and pre-clinical *in vivo* screening methods implemented in formulation assessment. The formulation and assessment approaches utilised are further described in the introduction below.

Bioenabling formulation approaches

At a high level, there are two strategies to deal with poor solubility and/or dissolution in drug development (Lohani et al., 2014);

1. Lead modification; ensuring good biopharmaceutical properties are incorporated into the molecule during the drug design

2. Formulation development; mitigating the problem through prudent selection of drug form, excipient and production methods

With the ever-increasing prevalence of lipophilic, poorly soluble compounds in drug development pipelines and the limited ability of lead modification to enhance molecule developability, the successful delivery of these challenging compounds will often rely on the use of novel bioenabling formulations, designed to enhance their *in vivo* solubility and/or dissolution (Hauss, 2007, Williams et al., 2013b, Butler and Dressman, 2010). Dissolution is considered the rate-determining step for absorption of poorly soluble and highly permeable (BCS class II) compounds and bioenabling formulations are frequently investigated for their potential to improve oral bioavailability of these molecules through increases either in the rate of absorption or in overall extent of bioavailability (Buckley et al., 2013). While changes in drug products generally have less influence over permeability, which tends to be an innate characteristic of a compound, there are cases where excipients have an impact on *in vivo* permeability, though these are rare (Butler and Dressman, 2010). The primary focus, however, has been on the development of bioenabling formulations, which enhance the solubility and/or dissolution of poorly soluble drugs and generate supersaturation, a metastable state where drug concentrations exceed the

8

equilibrium solubility in the medium, facilitating absorption. This is both due to these molecules being the most commonly observed class in drug development pipelines, and the fact that these molecules are the most amenable to formulation approaches designed to overcome their biopharmaceutical limitations. This has provided a focus for the development of bioenabling formulations, ultimately with the aim of ensuring BCS class II compounds will behave more like BCS class I compounds *in vivo* through enhancing dissolution and generating supersaturation. To exploit supersaturation as a strategy to improve intestinal absorption of poorly water-soluble drugs, the supersaturated state needs to be both generated and maintained. Guzmán et al. (2007) described this concept by using the term 'spring and parachute approach', as illustrated in figure 1-1. The ability of these formulations to generate supersaturation has been described as the 'spring' that enhances absorption and bioavailability (Brewster et al., 2008, Guzman et al., 2007). Generation of a 'spring' relies on reduction in the energy required for dissolving drug, for example by forming an amorphous drug form, or by presenting the drug in a pre-solubilised form, bypassing the need for dissolution altogether. Supersaturated solutions contain drugs at higher concentrations than their saturation solubility, and acts a driver of intestinal flux, facilitating absorption. However, supersaturated states tend to be inherently thermodynamically unstable and a potential risk of such strategies is the possibility of drug precipitation. Precipitation generally results in the formation of the energetically favourable crystalline form of a drug, which will have poor dissolution properties. Formation of such a crystalline form following formulation delivery will negate the favourable dissolution performance associated with formulating drugs in this manner. For this reason, bioenabling drug delivery systems often also include a

9

'parachute' which aims to stabilise and prolong the supersaturated state, be generating a metastable condition (Guzman et al., 2007). Such parachutes may include the use of precipitation inhibitors, or by utilising solubilising species such as lipids and surfactants to enhance solubility and reduce the maximum supersaturation ratio, maintaining the supersaturated state (Williams et al., 2013a, Xu and Dai, 2013).
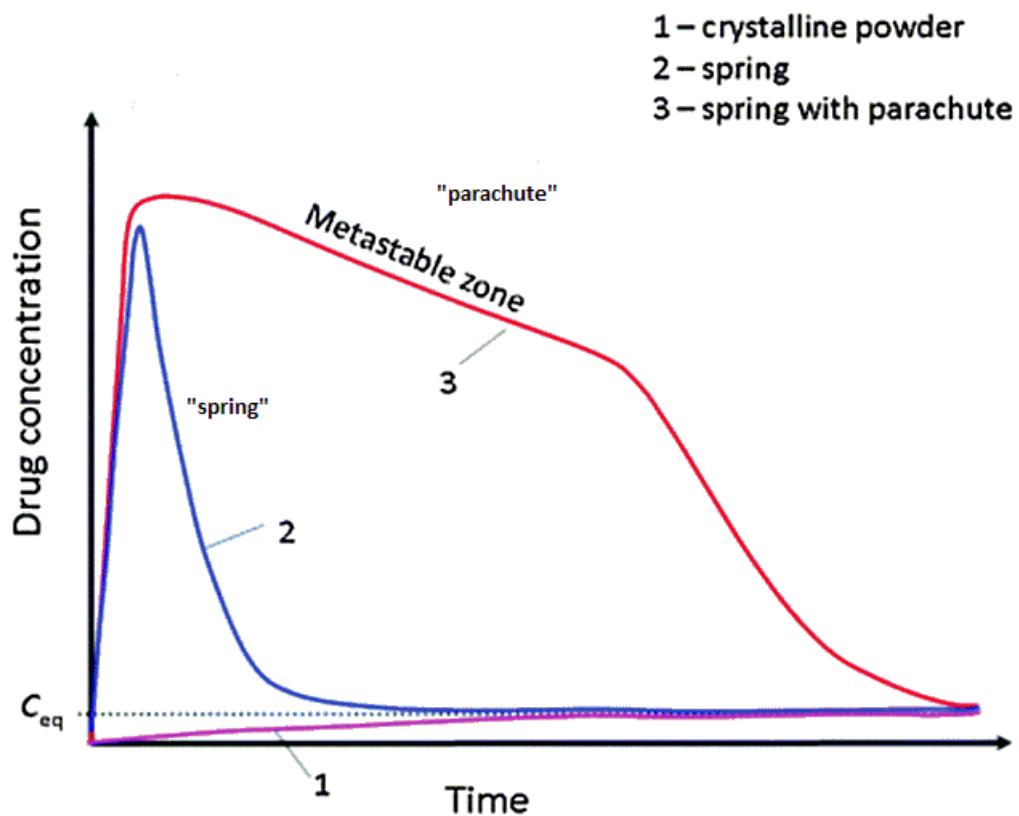

Figure 1-1 Schematic drug concentration–time profiles illustrating the spring and parachute approach of supersaturating drug delivery systems. Profile 1: dissolution of the most stable crystalline phase; profile 2: dissolution of a higher energy ''spring'' form of the drug in absence of precipitation inhibitors; profile 3: dissolution of a higher energy 'spring' form of the drug in presence of precipitation inhibitors that act as a ''parachute.'' C_{eq} represents the equilibrium solubility. Adapted from (Brouwers et al., 2009)

10

With this in mind, there has been much research in a wide range of formulation approaches designed to improve drug solubility and/or dissolution and potentially generate supersaturation. These approaches include those listed below and summarised in figure 1-2 (Williams et al., 2013b);

1. Salt formation

2. Optimising crystal habit/ using optimal polymorphs

3. Addition of co-solvents and/or surfactants

4. Complexation with cyclodextrins

5. Particle size reduction – including micronisation and nanonisation

6. Lipid based formulations

7. Amorphous solid dispersions

We have divided these approaches, very broadly, into two contrasting but complementary groupings, namely dissolution enhancing techniques and solubilising approaches. In practical terms, solubilising techniques will also lead to increases in dissolution rate, while it can also be difficult to definitively and exclusively classify these approaches to one group or the other. This is exemplified by the fact that nanonisation of particles straddles both classifications, where reduction in particle size to the nano-scale is expected to increase dissolution rate, but also has been shown to result in the formation of imperfections in crystals, leading to a corresponding increase in the kinetic solubility of dissolving particles (Shah et al., 2016, Chen et al., 2017).
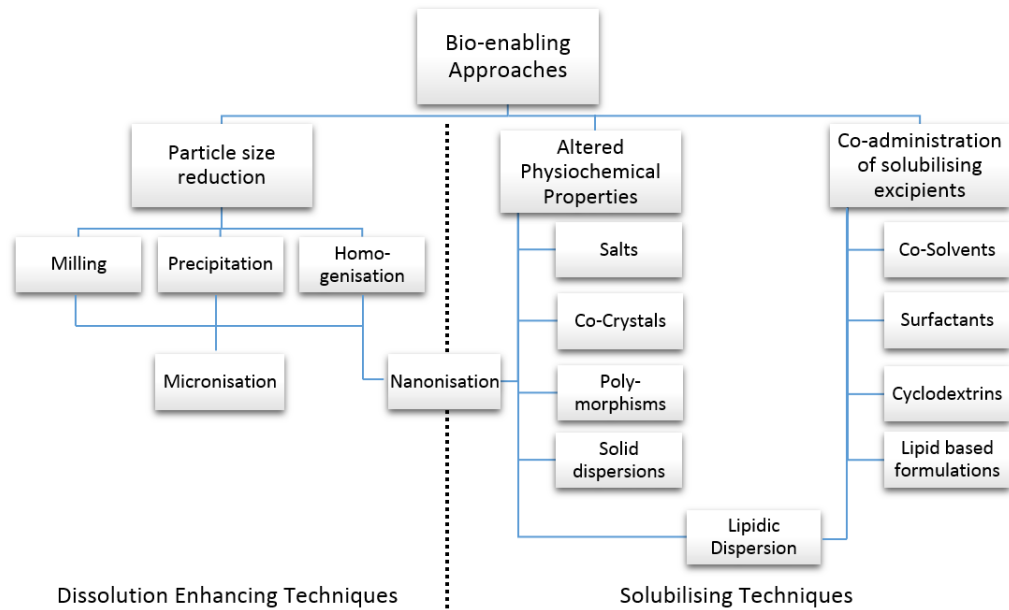
Figure 1-2 Approaches to formulation design for poorly water soluble drugs (PWSD)

One of the primary aims of the current thesis was the development and assessment of novel bioenabling formulations in the pre-clinical pig model. Two different formulation platforms were assessed during this thesis

- Lipid based formulations

- Mesoporous silica based solid dispersions

A brief introduction to these formulation platforms is provided here.

Lipid based formulations

One approach to bioenabling formulation development is the use of lipid-based formulations (LBF), where drug is co-formulated with exogenous lipids to enhance solubility and dissolution. LBF confer numerous biopharmaceutical advantages for delivering poorly soluble API, which are summarized in table 1-1 and discussed here

Table 1-1 Biopharmaceutical effects of oral lipid based formulations, adapted from (Constantinides and Wasan, 2007) and (Benet, 2013).

BCS class	% Marketed drugs	% New molecular entities	Biopharmaceutical effects of Lipid Based Formulations	Potential of Lipid based formulations
Class I High Solubility High permeability	40%	18%	Gut wall efflux↓	+/-
Class II Low Solubility High permeability	33%	54%	Solubilisation ↑ Permeability ↑ Efflux ↓ Lymphatic transport↑	+++
Class III High Solubility Low permeability	21%	22%	Permeability ↑ Enzymatic degradation↓ Efflux↓	+
Class IV Low Solubility Low permeability	6%	6%	Solubilisation ↑ Permeability ↑ Efflux ↓ Lymphatic transport↑	+++

LBF have long been investigated for their role in enhancing the absorption of PWSD. LBF include many different types of drug delivery systems, but particular interest has been shown in self-emulsifying drug delivery systems (SEDDS). SEDDS are combinations of digestible oils with surfactants, which spontaneously emulsify to form a stable emulsion on dispersion in the GIT and are of particular interest in the wider literature owing to the numerous commercially available examples, with Neoral® being the most well documented success story. Neoral® is a SEDDS formulation of cyclosporine which improved the oral bioavailability of this poorly soluble immunosuppressant. Additionally, the SEDDS formulation was less sensitive to food effects and reduced inter-individual variability (Mueller et al., 1994). Given the commercial success of such approaches, a SEDDS formulation approach was identified as a promising platform for assessment in this thesis.

Mechanisms of lipid mediated improvements in bioavailability

The original rationale for the investigation of LBF to improve absorption of PWSD was the observation that numerous drugs showed favourable increases in oral bioavailability when co-administered with food. Clinical reliance on co-administration with food, however, is inherently variable and unpredictable owing to variability in food ingestion and meal composition (Williams et al., 2013b). Focus instead shifted to identification of the physiological and biopharmaceutical properties underpinning this effect. Understanding these mechanisms has allowed co-administration of PWSD with formulated lipids, mostly derived from food oils, providing a predictable and reproducible route to the advantages of lipid co-administration. The primary methods by which LBF enhance absorption are described in this section and summarized in figure 1-3.
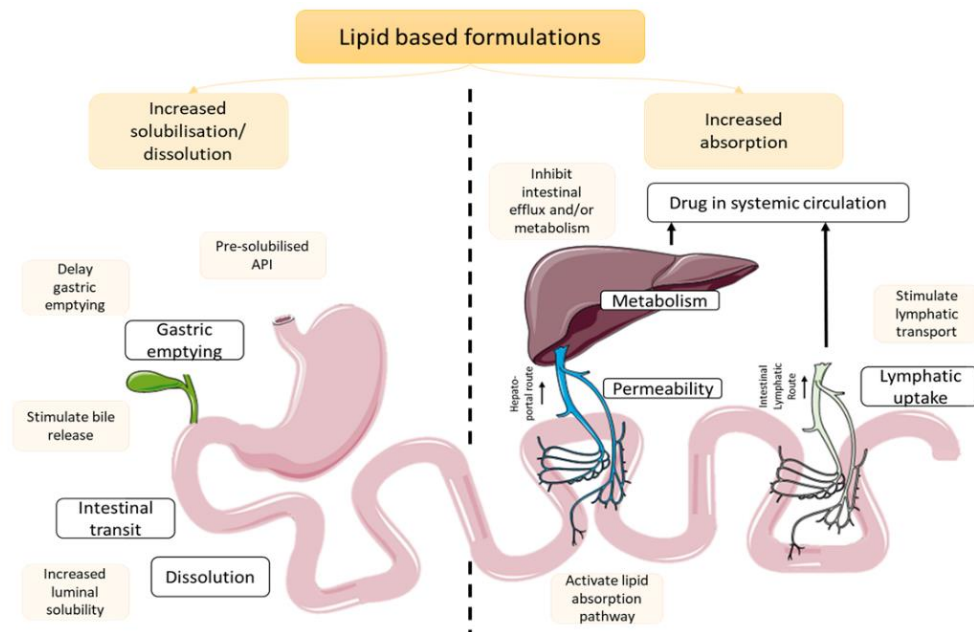
14

Figure 1-3 Schematic representation of the critical steps in oral drug absorption and the possible influences of lipid-based formulations. Solubility/ dissolution effects in left panel. Permeability/absorption effects in right panel. Adapted from (O'Driscoll and Griffin, 2008).

Increased drug solubilization and dissolution in the GIT

The ability to pre-solubilize drug in LBF, allowing delivery of a lipidic solution offers a clear advantage for drugs which display dissolution-rate limited bioavailability. Maintenance of solubilisation on formulation dispersion bypasses the drug dissolution step that is required when drug is administered in a crystalline solid-state (Mu et al., 2013). Additionally, the lipidic excipients – namely lipids and lipophilic surfactants – and co-solvents can also improve drug solubilization within the GIT (O'Driscoll and Griffin, 2008).

Increased intestinal permeability, reduced first pass metabolism and intestinal efflux

Lipids, surfactants and co-solvents, the principal components of LBF, have all been shown to impact intestinal permeability. In particular, the permeability enhancing effects of various bile salts, exogenous surfactants and end-stage lipid digestion

15

products are well known (Goole et al., 2010). These include increasing transcellular flux by promoting membrane solubilization and increasing membrane fluidity, inhibiting efflux transporters and intestinal enzyme activity and altering tight junction (TJ) integrity (figure 1-4). Promotion of lymphatic uptake will also result in avoidance of first pass metabolism (FPM).

Promotion of intestinal lipid absorption and lymphatic uptake

It is noteworthy that dietary lipids share many of the physicochemical properties that predispose PWSD to poor absorption, yet these dietary components are well absorbed. This occurs as a result of a highly efficient lipid absorption process (Gajjar et al., 2007). Dietary lipids stimulate the release of biliary salts and lipids, while also promoting secretion of lipase enzymes. These enzymes ultimately digest triglycerides to monoglycerides and fatty acids, which are solubilized in bile salt mixed micelles, from which absorption can occur. Once in the enterocyte, lipids enter the systemic circulation either *via* the portal blood or the intestinal lymphatic system (figure 1-4).

Drug absorption from LBF can be described as 'piggy backing' on the lipid absorption pathway. Co-administration of formulation lipids is intended to stimulate the same physiological response as dietary lipids and studies have shown that LBF can indeed generate at least a 'semi-prandial' response by stimulating gallbladder contraction and slowing gastrointestinal transit (Kossena et al., 2007, Christiansen et al., 2016).

Targeting of lymphatic uptake using LBF is somewhat more specific than attempting to exploit the lipid absorption pathway and is a function of both formulation design and the physicochemical properties of the drug. Inclusion of long chain lipids in a formulation is more likely to promote intestinal lymphatic absorption of highly

16

lipophilic drugs, *via* stimulation of triglyceride rich lipoproteins synthesis in intestinal cells. Specific drug characteristics favouring lymphatic absorption include high lipophilicity (e.g. log P > 5, solubility >50mg/g in triolein) (Lawless et al., 2015).

Figure 1-4 provides a useful summary of the mechanisms of transport from LBF into systemic circulation, highlighting these bioenabling mechanisms. Table 1-1 meanwhile provides a summary of the expected benefits of LBF for each class of BCS compounds.
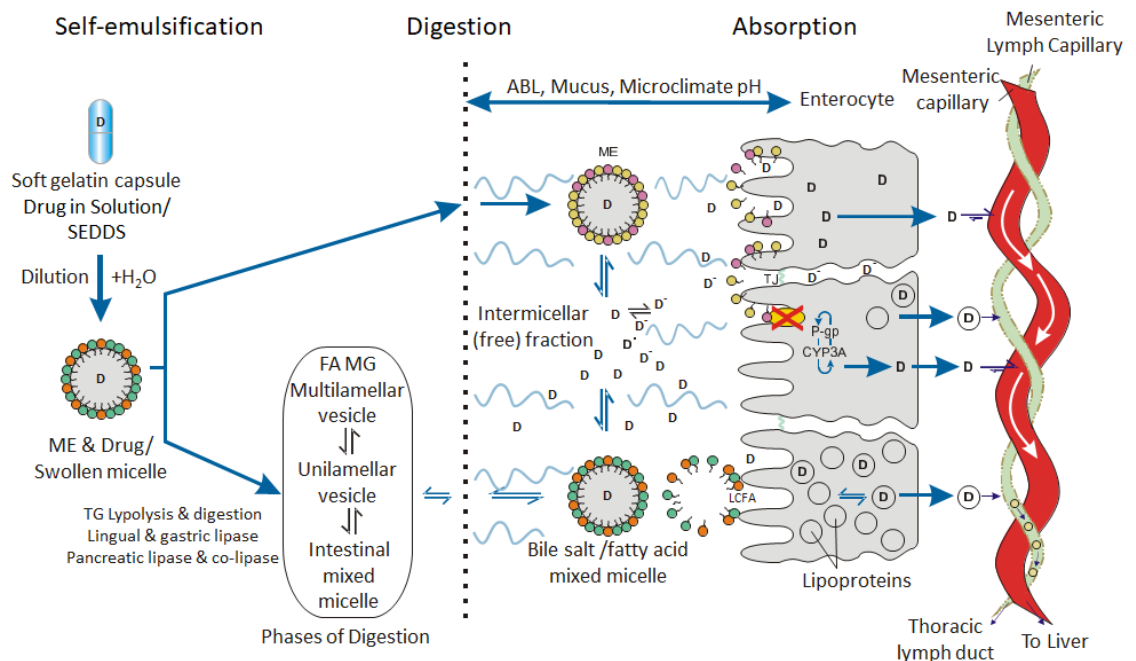

Figure 1-4 Schematic diagram of the mechanisms of intestinal drug transport from lipid-based formulations via the portal and mesenteric lymphatic routes. The main effects shown include: (A) increased membrane fluidity facilitating transcellular absorption, (B) opening of TJ to allow paracellular transport, (C) inhibition of P-gp and/or CYP450 to increase intracellular concentration and residence time, and (D) stimulation of lipoprotein/chylomicron production. ABL, aqueous boundary layer; D, drug; D−, ionized drug; FA MG, fatty acid monoglyceride; LCFA, long chain fatty acid; ME, microemulsion; SEDDS, self-emulsifying drug delivery systems; TG, triglyceride; TJ, tight junction, adapted from (O'Driscoll, 2002).

Summary

LBF have proved successful in enhancing the bioavailability of PWSD, with numerous commercial examples. The propensity for formulation lipids to enhance dissolution and solubilization in the GIT, stimulate physiological absorption pathways and facilitate permeation means that LBF are ideally tailored to overcome poor absorption characteristics of PWSD, and particularly with regard to eliminating food mediated changes in bioavailability. While current *in vitro* tools used in the assessment of LBF suitability and prediction of performance give insights into the likely fate of a formulation, direct *in vitro- in vivo* correlation (IVIVC) remains elusive. In particular, the complex relationship between solubilization, supersaturation and precipitation on formulation dispersion and digestion and the overall effect *in vivo* remains a significant area of interest. Establishing more biorelevant *in vitro* tests, the refinement of PBPK models and the increasing volume of preclinical *in vivo* data is aiding our understanding of the critical quality attributes (CQA) of LBF.

Thus, development of a novel LBF and its preclinical characterisation are among the aims of the current thesis. Given the significant interest in the ability of LBF to overcome food effect bioavailability, a particular emphasis is placed on eliminating food effect with a novel lipidic dispersion. The ability of *in vitro* and *in silico* tools to forecast pre-clinical *in vivo* performance in pigs is also assessed. These aspects of formulation development are further discussed in the proceeding sections of this introduction.

Mesoporous silica

Amorphous solid dispersions

In recent years, the use of amorphous materials, and amorphous solid dispersions (ASD) in particular, has gained considerable traction for solubility and dissolution enhancement of poorly water soluble drugs (Vo et al., 2013, Newman et al., 2015, Vasconcelos et al., 2007). Amorphous materials differ from crystalline compounds due to the lack of long-range order, leading to higher free energy. As a result, amorphous materials can display an increase in apparent solubility, even up to several orders of magnitude over the corresponding crystalline form, leading to generation of supersaturated state upon dissolution, providing an initial 'spring' to drive bioavailability (Guzman et al., 2007). However, one drawback of amorphous materials is the higher free energy associated with this metastable state can lead to recrystallization upon storage, or indeed that the supersaturation generated after dissolution can be transient and lead to precipitation of drug in a crystalline form, negating the solubility advantage achieved by formulating drug in this manner. Consequently, it is often necessary to stabilise formulations containing glassy, amorphous materials. While the traditional approach to stabilise amorphous materials is through their dispersion within inert polymer carriers, forming amorphous solid dispersions, mesoporous silica carriers are gaining increasing interest as alternative carriers to stabilise such formulations (Newman et al., 2015, Vo et al., 2013).

Mesoporous silica drug delivery systems

Mesoporous silica is a form of silicon dioxide, which possesses pores in the range of 2-50nm and it is possible to control the pore size and architecture during the manufacturing process (Maleki et al., 2017). The porous structure leads to vast increases in surface area (700-1000m^2/g), relative to non-porous material, facilitating drug loading onto the silica surface. In particular, ordered mesoporous silicas, which possess uniform pore size, shape and volume, have proven beneficial in drug delivery and in enhancing the dissolution of PWSD (Van Speybroeck et al., 2009, Kiekens et al., 2012). The characteristics of the mesoporous silica material itself plays a crucial role in determining the drug loading and release characteristics of the formulation. In particular the pore size, volume and geometry, level of drug loading, surface functionalisation and overall particle surface area play major roles in controlling release characteristics (McCarthy et al., 2016). By controlling pore size and volume as well as the surface chemistry, the formulation can be tailored for each drug molecule with regard to its physicochemical characteristics. The optimised preparation can maximise drug loading and facilitate long-term stability *via* inhibition of recrystallization within the limited pore space (Salonen et al., 2008, Maleki et al., 2017). Mesoporous silica formulations possess a number of advantages in improving dissolution of poorly soluble drugs, which have been the focus of significant research over the last two decades, culminating in the first human study in 2016. This study demonstrated an improvement in fenofibrate bioavailability, indicating the promise of mesoporous silica formulations in drug delivery (Bukara et al., 2016b). Some of these properties are briefly discussed here.

Factors affecting drug loading, release and stability

Adsorption of drug onto a mesoporous silica carrier has a number of advantages for enhancing drug dissolution. The ordered mesoporous structure and high porosity of silica allows high drug loads to be adsorbed (Vialpando et al., 2011, Singh et al., 2011). Drug molecules adsorb to the silica surface in a noncovalent manner, principally through electrostatic interaction, hydrogen bonding or van der Waal's forces, which are easily broken on exposure of the formulation to an aqueous environment, enabling release of drug in its molecular form and facilitating absorption. The drug can exist in an amorphous or molecularly dispersed state on the silica surface, thus displaying higher apparent solubility and dissolution rate compared to the crystalline substance (Hancock and Zografi, 1997). Although amorphous materials can display thermal instability, adsorption onto the mesoporous silica has proven effective in stabilising amorphous systems, and the long-term stability of these formulations has been repeatedly demonstrated (McCarthy et al., 2016, Van Speybroeck et al., 2009). Adsorption to the silica surface generates a physical stabilisation effect, due to a decrease in the free energy of the drug/silica system, reducing the tendency to crystallise (Morris et al., 2001). Secondly, size-confinement of molecularly dispersed amorphous materials can also lead to stabilisation of the system. Drug molecules in the mesopores are constrained to such an extent that they cannot reach a critical nucleation size, therefore preventing crystal formation and growth (Alcoutlabi and McKenna, 2005). The critical pore size at which crystal growth is prevented is generally observed to be approximately ten to fifteen times the molecular size (Maleki et al., 2017, Sliwinska-Bartkowiak et al., 2001, Rengarajan et al., 2008). Along with controlling pore size and volume, it is also possible to functionalise the silica

21

surface with differing functional groups, such as amino groups or alkyl chains of varying lengths, in order to alter drug loading and/or release (Balas et al., 2006, Doadrio et al., 2006).

Supersaturation and precipitation in mesoporous silica formulations

The presence of adsorbed drug in a stabilised amorphous or molecularly dispersed state on the surface of mesoporous silica is useful in enhancing the solubility/dissolution behaviour of PWSD, and can be used to enhance drug bioavailability (Bukara et al., 2016b, Van Speybroeck et al., 2011). The favourable dissolution and solubility behaviour associated with these formulations can be used to generate supersaturation, where the concentration of solute within the solution is above the thermodynamic equilibrium solubility, favouring absorption, providing the 'spring' for the supersaturated state. A drawback of using mesoporous silica materials for stabilisation of amorphous systems is that the more conventional polymeric matrices may also have inhibitory effect on precipitation, even after drug release from the polymeric carrier (Laine et al., 2016). To counteract this problem, an increasing body of research has focused on the co-formulation of mesoporous silica formulations with polymeric precipitation inhibitors (Laine et al., 2016, Dressman et al., 2016, Van Speybroeck et al., 2010b).

Polymeric precipitation inhibitors

One method to create a 'parachute' is the inclusion of a polymeric precipitation inhibitor (PPI) as a functional excipient to slow the rate of precipitation (Van Speybroeck et al., 2010b, Warren et al., 2010, Laine et al., 2016). The use of PPIs has previously been established in the development of conventional solid dispersion formulations, as well as in other formulation approaches including LBF (Yamashita et al., 2003, Gao et al., 2003). PPIs aim to maintain drug in a metastable supersaturated state over a period of time that is sufficient to allow absorption (Warren et al., 2010). In general PPIs are effective only at delaying the rate of precipitation, and do not alter the equilibrium solubility. PPIs are thought to slow precipitation by both preventing nucleation, which is a pre-requisite to crystal formation precipitation, and prohibiting crystal growth on nuclei which have already been formed. A wide range of potential mechanisms have been identified, which can be broadly classified into two distinct categories; (1) altering solution bulk properties, such as surface tension and diffusivity, preventing nucleus formation and (2) adsorption to crystal interface, growth terraces and imperfections, principally through hydrogen bonding and hydrophobic interactions, preventing crystal growth through steric hindrance (Machefer et al., 2008, Gao et al., 2009, DiNunzio et al., 2008, Ilevbare et al., 2012, Xu and Dai, 2013). A wide range of polymeric precipitation inhibitors exist, including polyvinylpyrrolidine (PVP), hydroxypropyl methylcellulose (HPMC), hydroxypropyl methylcellulose acetate succinate (HPMCAS) and copovidone (PVPVA) (Dressman et al., 2016). A point to consider is the lack of a 'one size fits all' approach to choosing a PPI. While "there are likely to be common functional attributes of 'good' PPIs" (Warren et al., 2010), effective screening remains essential and choice of an

23

appropriate PPI is crucial. This is exemplified by the studies by Van Speybroeck *et al.* (2010) and Dressman *et al.* (2016). These studies had contrasting findings on the relative performance of the cellulosic derivatives, HPMC and HPMCAS, on precipitation indicating there is still a ways to go fully elucidating the mechanistic understanding of PPI performance, and polymer performance may well be compound specific (Bevernage et al., 2011, Dressman et al., 2016, Van Speybroeck et al., 2010b).

Summary

Mesoporous silicas have demonstrated excellent properties for the enhancement of drug dissolution and oral bioavailability of a variety of poorly water-soluble BCS II compounds and while a commercial preparation has yet to be realised, a first, human proof of concept study has recently demonstrated the realistic prospect of such a formulation (Bukara et al., 2016b). The ability of stabilised amorphous drug loaded onto mesoporous silica to generate a supersaturation and improve oral bioavailability has been repeatedly demonstrated, and the use of precipitation inhibitors to stabilise such systems has also been addressed. However, there remains gaps in the knowledge regarding the *in vitro* and *in vivo* performance of such formulations (McCarthy et al., 2016). One of the aims of the current thesis is the *in vitro* and *in vivo* assessment of such a formulation to determine its ability to overcome dissolution/ solubility limitations of a model PWSD, while also attempting to link *in vitro* and *in vivo* performance.

Food effects on bioavailability and formulating away the 'food effect'

The concomitant administration of oral dosage forms with food can have a significant impact on drug pharmacokinetics and bioavailability relative to the fasted state. With oral drug delivery continuing to be the method of choice for drug administration, understanding the effects food has on the biopharmaceutical aspects of drug delivery is key to the drug development process as well as the effective and rational use of medicines in the clinical setting (Fleisher et al., 1999, Abuhelwa et al., 2017). As previously mentioned, the original rationale for designing LBF was the observation of increased oral bioavailability for many drugs when they were taken with food. While there are clinical and commercial preferences to formulate drug products to overcome food effects, co-administration with food, as a bioenabling approach, has been utilised clinically through the provision of 'label claims' with regard to food intake for many drugs, where patients are instructed to take these medications in a specific prandial state, quite often with the aim of enhancing bioavailability. In this regard, co-administration with food can be considered a bioenabling technique in its own right. The use of this approach is widespread, with research carried out by this group suggesting that approximately 40% (67 of 157 products identified; 42.68%) of medicines licensed by the EMA and FDA since January 1st, 2010 display a significant food effect or have been licensed with a label restriction with regard to dosing with or without food (O'Shea et al., 2018). The effects of food on drug bioavailability and the mechanisms which underpin them are described briefly here.

What is a food-effect?

In its simplest terms, food effects on drug absorption are observed when the rate and/or extent of drug bioavailability is altered when a drug or drug product is administered in fed state, compared to the fasted state. The clinical effects and significance of food effects on absorption are generally assessed with regard to the rate and extent of bioavailability – as measured by peak plasma concentrations (C_{max}), time to peak plasma concentration (T_{max}) and the total extent of bioavailability (area under the curve; AUC) (Fleisher et al., 1999). Welling classified food drug interactions into five categories causing (Welling, 1989);

- Reduced extent of bioavailability

- Delayed rate of absorption

- Increased extent of bioavailability

- Accelerated rate of absorption

- No effect

With regard to clinical significance, the most crucial aspect of food effect is generally considered to be the extent of bioavailability change, and the terms 'positive food effect' and 'negative food effect' have been coined to describe either an increase or decrease in the overall extent of bioavailability, respectively (Fleisher et al., 1999). FDA guidance defines that a food-effect is established if the 90% confidence intervals for the ratio of population geometric means, based on log-transformed data, for either $AUC_{0\rightarrow\infty}$ or C_{max} fall outside the 80-125% bioequivalence limits relative to the reference, i.e. the same formulation administered in the fasted stated (FDA, 2002). The fed state represents dosing post ingestion of a high fat, FDA standard breakfast,

containing 800 – 1000 kcal with approximately 50% of total calories coming from fat, to maximise potential for demonstrating a food effect (FDA, 2002).

Figure 1-5 illustrates the key steps in drug absorption and bioavailability and indicates how food influences these processes.
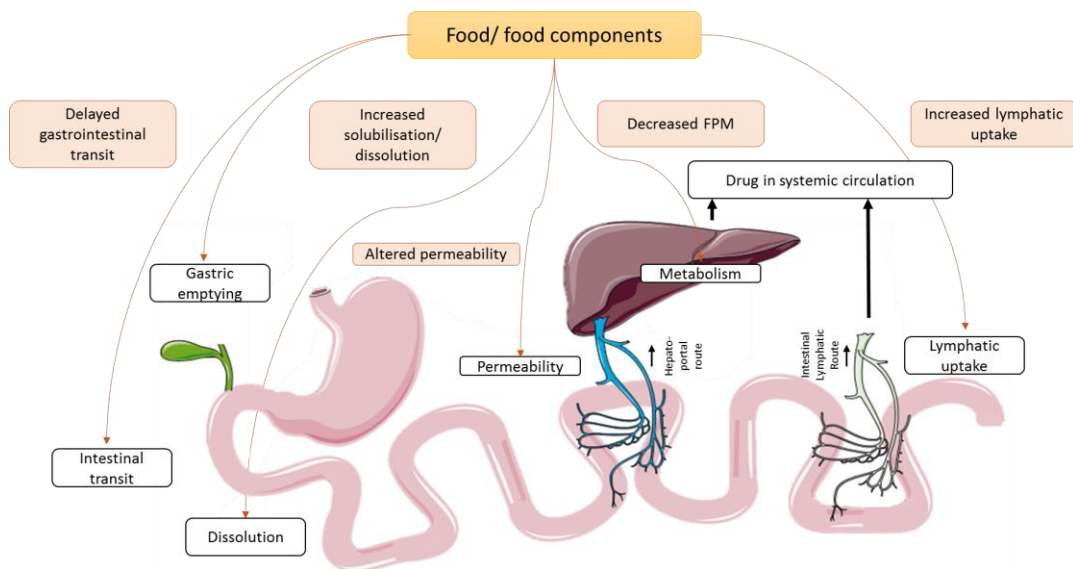

Figure 1-5 Schematic diagram of critical steps in drug absorption and influence of food and food components; FPM: first pass metabolism

Mechanisms underlying the food effect

Drug absorption via the oral route is a function of the interplay of various complex biopharmaceutical processes, namely (i) drug molecular and physicochemical properties, (ii) formulation characteristics, (iii) the physiological changes of the gastrointestinal tract induced in the fed state and (iv) the physical chemical changes in the composition of the gastrointestinal fluid (Fleisher et al., 1999). The Biopharmaceutical Classification System (BCS) and Biopharmaceutical Drug Disposition Classification system (BDDCS) provide a useful predictor of potential food

27

effects based on drug physicochemical properties, as summarised in figure 1-6 (Wu and Benet, 2005, Amidon et al., 1995). The anticipated effects are predicted by the most likely limiting factor for bioavailability, namely solubility or dissolution for BCS/BDDCS class II compounds, permeability for class III compounds, or a combination thereof for BCS class IV compounds. An overall delay in T_{max} and reduced C_{max} for highly bioavailable compounds can be associated with a delayed gastric emptying (Custodio et al., 2008). While this tool does not capture all the potential effects of food, it is the most widely utilised simple tool to predict food effect behaviour, and is estimated to be accurate in approximately 70% of cases (Benet, 2013).

Figure 1-6 Predicted effect of high fat meals by BCS/BDDCS class. Adapted from Custodio et al. (2008)

Drug absorption is inherently variable, owing to both inter- and intra-individual variability in the physiology of the GIT. When considering the gut physiology McConnell *et al.* have stated that there is 'no such thing as an average person'(McConnell et al., 2008), and despite regulatory guidance, equally there is no such thing as a standard meal (FDA, 2002). The purpose of FDA guidance is to provide a standard for bioavailability and bioequivalence studies, where the likelihood of observing a food effect is maximised. However, this is not always reflective of the fed state for patients, which adds further to the variability and complexity of absorption and drug product performance.

In the fed state the physicochemical composition of the gastrointestinal fluid, including its volume, pH, osmolality, surface tension, hydrodynamics and overall composition change (Abuhelwa et al., 2017, Clarysse et al., 2009b, Abuhelwa et al., 2016a). Modulation of other physiological functions, such as gastrointestinal transit, enzymatic and intestinal transporter activity and endo- and exocrine secretions also occur in the fed state (Won et al., 2012, Custodio et al., 2008, Abuhelwa et al., 2016b, Varum et al., 2013). There are a number of additional factors that may influence absorption from oral dosage forms in the fed state, with the most pertinent aspects summarised in figure 1-7.
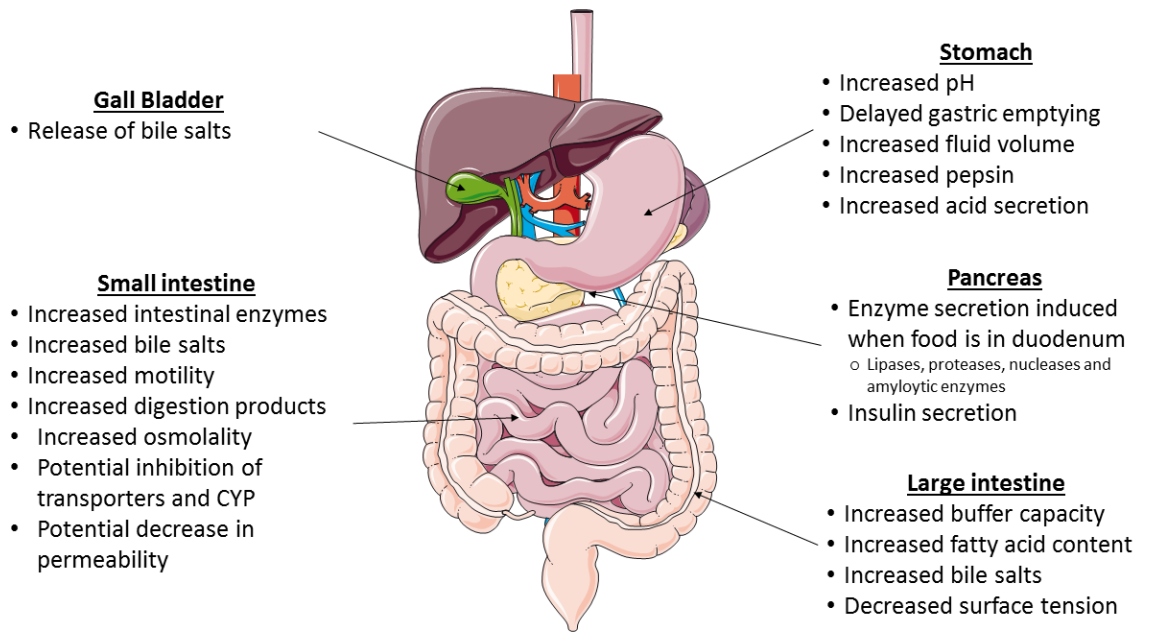
Stomach
- Increased pH
- Delayed gastric emptying
- Increased fluid volume
- Increased pepsin
- Increased acid secretion

Gall Bladder
- Release of bile salts

Small intestine
- Increased intestinal enzymes
- Increased bile salts
- Increased motility
- Increased digestion products
- Increased osmolality
- Potential inhibition of transporters and CYP
- Potential decrease in permeability

Pancreas
- Enzyme secretion induced when food is in duodenum
  - Lipases, proteases, nucleases and amyloytic enzymes
- Insulin secretion

Large intestine
- Increased buffer capacity
- Increased fatty acid content
- Increased bile salts
- Decreased surface tension

Figure 1-7 Summary of human physiological changes in the fed state (adapted from (Varum et al., 2013))

Mechanisms of food effect

As has been mentioned above, food has a complex and significant effect on the physiology of the gastrointestinal tract and the physicochemical properties of gastrointestinal fluid, which in turn can have a significant effect on drug absorption. These effects are dependent on both the physicochemical properties of the drug, principally solubility, pKa and LogP/logD, and formulation characteristics, including release and disintegration of solid dosage forms (Mullertz, 2010, Gu et al., 2007, Singh, 2005). For the purposes of this introduction, the focus will predominantly be on immediate release and bioenabling formulations, the mechanisms by which food causes these changes in bioavailability are discussed here and summarised in table 1-2.

Positive food effects

The principal cause of positive food effects is the increase in dissolution and solubilisation of PWSD in the fed state. The release of bile salts and the presence of exogenous solubilising species, such as ingested lipids and their digestion products serve to enhance solubilising capacity of gastrointestinal fluid (Augustijns et al., 2014, Stappaerts et al., 2014, Geboers et al., 2016, Clarysse et al., 2011, Clarysse et al., 2009a, Di Maio and Carrier, 2011). For drugs which are dissolution rate, rather than solubility limited, the increased gastric residence time also can improve bioavailability, while the increase in gastric pH may result in improved solubility and dissolution of weak acids. In practical terms, it is difficult to isolate the impact of any one of these factors, which work synergistically to increase solubility and dissolution of PWSD.

The inhibition of intestinal transporters can play a role in enhancing bioavailability of certain drugs. Wu and Benet have demonstrated that for BCS class II compounds efflux transporters predominate, and that for these compounds transporter inhibition is likely to improve bioavailability (Elgart et al., 2013, Wu and Benet, 2005, Custodio et al., 2008, Benet et al., 2004).

Reduction in first pass metabolism in the fed state can also lead to increases in bioavailability and this can occur through numerous mechanisms including altered blood flow, increased lymphatic uptake and reduced enteric metabolism. Food intake is associated with an increase in splanchnic blood flow by as much as 60% depending on the volume and nature of the meal. This allows drug to bypass the liver, while the increase in hepatic blood flow may also reduce the first pass effect for drugs which

display low to moderate clearance (Melander and McLean, 1983, Marasanapalle et al., 2011, Liedholm et al., 1990). Co-administering lipophilic drugs with food allows efficient absorption of these molecules with dietary lipids, *via* lipid absorption pathways, while particularly lipophilic drugs (logP>5) can also show significant lymphatic uptake (Di Maio and Carrier, 2011, Charman and Stella, 1986, Lawless et al., 2015). This can increase the systemic absorption by both increasing the fraction escaping the gastrointestinal lumen and reducing the first pass effect.

The inhibitory effect of food on meal components on CYP3A4 is also a significant contributor to the reduction of enteric drug metabolism and increased bioavailability in the fed state. Inhibition of CYP3A metabolism by grapefruit juice has been widely associated with increases in bioavailability and subsequent increases in adverse events for a wide range of pharmacologically diverse compounds (Custodio et al., 2008, Gibbs and Hosea, 2003). While other fruit juices and other food components, including teas and alcoholic beverages, as well as high fat meals generally have been implicated in reduced enzymatic activity, though the clinical implications of such interactions have not yet been extensively characterised (Won et al., 2012).

Negative food effects

Negative food effects encompass both reduced and delayed drug absorption. With regard to delayed absorption in the fed state, this often occurs for immediate release preparations without a corresponding reduction in overall bioavailability. The main mechanism by which this occurs is delayed gastric transit in the fed state. This manifests itself as a prolonged T_{max}, which may or may not be accompanied by a reduction in C_{max} or a significant lag time. For medicines which are chronically dosed

and where overall exposure, rather than peak plasma levels, mediate pharmacodynamic action, this is unlikely to result in clinically meaningful effects (Schmidt and Dalhoff, 2002).

Decreased absorption in the fed state results in a reduction in AUC, along with a reduction in C_{max}, and can lead to sub-therapeutic plasma levels and loss of efficacy. The most common causes of reduced bioavailability in the fed state are direct physicochemical interactions between drugs, or drug products, and food. One potential cause of this effect is the reduced diffusivity of drug in the viscous postprandial upper GIT. The increased viscosity can result in either inhibition of disintegration of a dosage form, preventing drug release, or hindering diffusion of drug to the absorptive membranes of the GIT (Radwan et al., 2014, Yildiz et al., 2015, Kelly et al., 2003, Cole et al., 2004). This can be problematic for poorly permeable drugs, particularly those with narrow absorption windows, as by the time viscosity has reduced in the distal gut, the absorption window has been traversed and absorption will be reduced (Radwan et al., 2012, Radwan et al., 2013, Radwan et al., 2017). This effect is amplified by viscous meals or those high in dietary fibre (Radwan et al., 2012, Rodin and Johnson, 1988). A second direct mechanism by which food can hinder drug absorption is by binding of drug with food components (Gertz et al., 1995, Schmidt and Dalhoff, 2002). This is prevalent in the case of polyvalent cations, which are abundant in dairy products (Leyden, 1985, Neuvonen et al., 1991, Schmidt and Dalhoff, 2002, Wallace and Amsden, 2002, Polk, 1989).

Physiological factors can also play a role in negative food effects, especially in the case of drugs displaying instability and possibly acid lability in the GIT. Prolonged

gastric residence can result in increased degradation of these molecules, though in the case of acid labile drugs the effect may be somewhat mitigated by the increase in gastric pH (Jones et al., 2006). Food can also result in alterations in absorption through altering both passive permeability and active transport. The presence of increased lipids and bile salts in the fed state can result in a decreased free fraction of drug, causing a reduction in permeability (Holmstock et al., 2013, Sugano et al., 2010, Kataoka et al., 2012, Singh, 2005, Stappaerts et al., 2014). While for poorly soluble drugs, this is generally more than compensated for by increases in solubility, highly soluble and poorly permeable compounds may display reduced absorption in this case.

The inhibition of uptake transporters may also result in negative food effects. For poorly permeable drugs, the inhibition of these transporters may result in a reduction in absorption, as these compounds are often reliant on the action of uptake transporters. The general inhibition of intestinal transporters observed in the fed state is therefore likely to reduce the bioavailability of BCS class III compounds. Care is needed, however, when applying this rule of thumb, as class III compounds may be candidates for both uptake and efflux transporters and the relative inhibition of either uptake or efflux transporters, or the extent to which a specific molecule will be a substrate for each particular class can determine the overall effect of bioavailability (Custodio et al., 2008).

The events described here are summarised and examples of drugs affected by the various mechanisms are provided in table 1-2.

Table 1-2 Summary of physiological mechanisms and biopharmaceutical aspects underpinning the food effect

Physiological mechanism	Biopharmaceutical aspects	Effect on Drug exposure	Example(s)
Increased pH in stomach	Solubility and dissolution of ionisable compounds can be altered	Increases AUC and Cmax for weak acids Decreases AUC and Cmax for weak bases	Cefuroxime Dipyridamole, indinavir
Increased concentration of solubilising species e.g. bile salts, lipid digestion products	Solubilisation of poorly water soluble drugs increases	Increases AUC and C_{max}	Fenofibrate Alectinib Danazol
Increased splanchnic blood flow	Saturation of liver enzymes and avoidance of FPM	Increases AUC and C_{max}	Propranolol; Tacrine; Dronedarone
Inhibition of gastrointestinal Cytochrome P450 – e.g. with Grapefruit juice	Fraction of drug escaping gut metabolism increases	Increases AUC and C_{max}	Felodipine; Ciclosporin; Atorvastatin
Inhibition of intestinal absorptive and efflux transporters	Fraction of drug subject to either absorptive or efflux transport is reduced	Increases AUC and Cmax for drugs subject to efflux Decreases AUC and Cmax for drugs which require uptake transporters	Ganciclovir Fexofenadine Talinolol
Delayed gastric emptying	Presence of food in stomach delays transit of drug to small intestine	Increases T_{max}, can decrease C_{max}, may cause T_{lag}	Widespread NSAIDs Paracetamol
Increase in viscosity of intestinal fluid	Reduction in water diffusivity, increase in luminal viscosity, slower water penetration of dosage form, increased disintegration time	Increases T_{max}, may reduce C_{max} and F, may cause T_{lag}	Chlorothiazide, Metformin

Summary

Despite the increased awareness of the negative clinical impact of food effects on bioavailability and the strict regulatory guidance regarding the appropriate testing of new medicinal products in the fed and fasted states there appears to be an ever-increasing challenge of food mediated alterations in drug bioavailability, likely reflecting the increasing prevalence of PWSD in drug development pipelines. While there has been increasing understanding and development of improved drug delivery technologies, there remains an overall lack of appreciation of the scale of the food effect challenge, as evidenced by the fact that over 40% of new medicines display significant food effects or possess a label claim in respect of dosing with regard to food intake. This has had a knock-on effect in the clinic, where the success or commercial advantage of compounds can be affected, particularly with antipsychotic and oncological preparations.

Formulating compounds to overcome food effect remains largely empirically driven, with only sporadic case studies for individual compounds published. While the presence or absence of food effects is unlikely to be a key driving factor in early formulation development, it can be a critical factor when entering the clinic. In the absence of large databanks of formulation design studies in easily obtainable literature, greater use of mechanistic and *in silico* approaches will be central to enhancing our ability to discriminate between formulations likely to overcome food-mediated alterations in drug bioavailability. Thus, the development of a novel formulation to overcome food effect, along with its preclinical *in vitro, in vivo* and *in silico* assessment are among the primary aims of this thesis.

Pre-clinical formulation screening

With the increasing need to develop novel formulation technologies to overcome the problems observed with increasing lipophilicity of development pipelines, there is consequently a greater need for efficiency in formulation development, and in particular in formulation assessment. Assessment of these novel drug/ formulation combinations involves a range of screening methods and *in vitro* techniques, before ultimately being assessed *in vivo*. Traditionally this development process has been largely empirically driven, resulting in an inefficient, iterative, 'trial and error' based approach involving screening of range of bioenabling formulations, before eventually selecting the most appropriate candidate formulation for clinical trials. However, this process is both inefficient as multiple formulation technologies are often developed and assessed in parallel and presents ethical implications as parallel development can lead to excessive pre-clinical bioavailability screening (Lennernas et al., 2014, Kuentz et al., 2016).

Overcoming this limiting factor involves greater use of predictive biopharmaceutical tools to model the interplay of various drug, formulation and physiological properties, with the ultimate goal of forecasting the ability of formulation technologies to improve oral bioavailability (Kawabata et al., 2011, Pandey et al., 2014). This will involve moving away from empirically driven development programs, toward a Quality by Design (QbD) approach, utilizing an improved biopharmaceutical toolkit based upon a sound scientific understanding of *in vivo* behaviour. Enhanced predictive capacity earlier in the drug development process can greatly reduce early risk, improve developability assessment of candidate drugs and potentially even

result in reduction of animal studies (Lennernas et al., 2014). The implementation of these enhanced biopharmaceutical tools will involve an integrated approach with a combination of physicochemical measurements, *in vitro* tests, *in vivo* studies and *in silico* models. By enhancing the mechanistic understanding of biopharmaceutical and absorptive processes efficient screening will remove bottlenecks in drug development improving overall efficiency (Kostewicz et al., 2014b). There is a need to assess and validate the predictive capacity of current methods of formulation assessment, and also design and implement optimized, novel laboratory assessments and *in silico* models that will better predict the biopharmaceutical performance where current approaches are found wanting (Flanagan et al., 2016).

The predictive capacity of new tools depends on their ability to simulate the dynamic gastrointestinal environment, particularly with regard to gastrointestinal transit, *in vivo* dissolution and/or precipitation and intestinal absorptive flux, incorporating the effects of the solubility- permeability interplay (Kostewicz et al., 2014b, Selen et al., 2014). To this end, there has been significant focus in the last number of decades and designing and validating improved biorelevant, biopharmaceutical tools for formulation assessment and prediction of *in vivo* performance. These approaches include the introduction and refinement of *in vitro* permeability models, development of biorelevant dissolution media and *in silico* PBPK models for integration of *in vitro* data and prediction of GI drug absorption. A brief overview of the *in vitro*, *in silico* and *in vivo* approaches implemented and assessed is provided below.

In vitro **screening**

The primary method of lab-based evaluation of oral solid dosage forms is by means of drug release testing, predominantly by means of *in vitro* dissolution testing, but also encompassing other methods such as disintegration and lipolysis. The link between drug release testing and *in vivo* performance has been recognized for well over 100 years. While traditional pharmacopoeial setups have proved useful in the quality control (QC) lab, both the apparatus and the media used in these compendial systems are far from optimized for dosage form development and predictive evaluation (Kostewicz et al., 2014b). This has led to significant development of these approaches over the past three decades, which is briefly summarised here.

Choice of media

One of the most crucial factors in biorelevant solubility and dissolution testing is the choice of dissolution medium when attempting to simulate the conditions of the intestinal lumen. While traditional compendial media have proven useful for QC purposes, they lack the biorelevance desired for the design of predictive biopharmaceutical dissolution tool. An alternative approach would be to use human and/or pre-clinical animal gastrointestinal aspirates for solubility and dissolution assessment. While such studies have been carried out, the obvious practical difficulties and ethical implications of using this approach on a routine basis has meant alternative approaches have been developed (Diakidou et al., 2009, Fagerberg et al., 2015, Augustijns et al., 2014). In order to be more predictive of *in vivo* performance and representative of the conditions in the intestinal lumen, the importance of using synthetic, simulated biorelevant media was first proposed in

1998 (Dressman et al., 1998). These media aim to provide an easily obtained and reproducible method of mimicking the physicochemical properties of human gastrointestinal fluid. Considerable efforts have been made in the interim to both characterize the luminal contents of the upper GI tract (Vertzoni et al., 2008, Kalantzi et al., 2006, Koziolek et al., 2013, Schneider et al., 2016, Clarysse et al., 2009b) and to design improved media have been proposed (Vertzoni et al., 2005, Vertzoni et al., 2010, Vertzoni et al., 2004, Jantratid et al., 2008, Fuchs et al., 2015, Markopoulos et al., 2015). This has provided pharmaceutical scientists with a wide array of different media, representative of different prandial conditions in both the stomach, small intestine and colon, along with 'snapshot' media, which are reflective of specific post-prandial timepoints in each region. Other specialist simulated media, such as that suitable for use in permeability assays, in lipolysis experiments or media with a modified viscosity for assessing disintegration or diffusion related effects have also been developed (Wuyts et al., 2015a, Wuyts et al., 2015b, Markopoulos et al., 2014, Klein et al., 2004, Cvijic et al., 2014, Williams et al., 2012, Zangenberg et al., 2001).

While the choice of media will ultimately depend on the particular aspect of the biopharmaceutical drug delivery process under investigation, Markopoulos et al. (2015) have recently suggested a hierarchical approach to choose dissolution media based on level of biorelevance required (figure 1-8). While for drugs which display high solubility and a low dose; solubility ratio (BCS class I and III compounds) a simple aqueous buffer system (Level I biorelevant media) may be sufficient to assess pH related effects. For poorly soluble and lipophilic drugs more complex media is recommended which includes bile salts and products of digestion (Level II biorelevant

media). The authors of this work suggest that Level III biorelevant media, which also includes dietary proteins, enzymes and simulates the increased viscosity of luminal contents in the fed state is not routinely used but may be necessary for drug or formulation specific considerations. Such examples include lipolysis testing for lipid based formulations, assessment of the impact of increased viscosity on modified release dosage forms or the effect of degradation on peptide based therapies (Markopoulos et al., 2015). While the availability of these media is a significant benefit for development scientists, limitations and drawbacks remain. Specifically, these media represent a snapshot of the luminal conditions in the upper intestine and cannot replicate the rapid and dynamic changes in the conditions along the intestine. The inter- and intra-individual variability in GI composition can be considerable, questioning the reliability of using a single medium. While, in general, the approaches involving biorelevant media represent an improvement over traditional compendial media in terms of predicting *in vivo* performance, there remains a of lack systematic validation of this approach.

Levels of simulation of luminal composition

+ dietary proteins, enzymes (not digestion products), viscosity effects

Level III

+ bile components, dietary lipids, lipid digestion products, osmolality

Level II

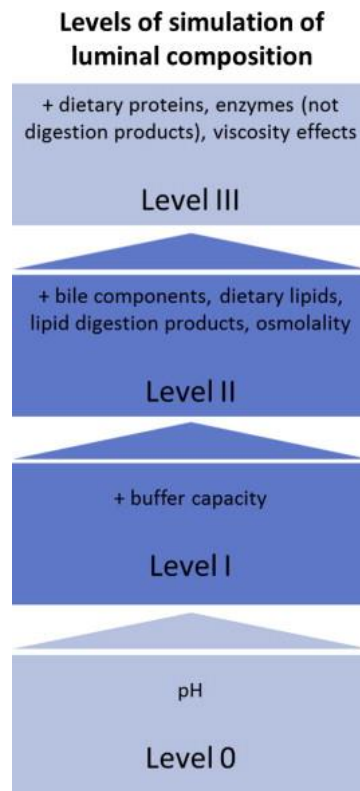+ buffer capacity

Level I

pH

Level 0

Figure 1-8 An overview of the four levels of biorelevant media recommended for the simulation of the luminal environment during development of oral formulations (Markopoulos et al., 2015)

Choice of apparatus

A second key factor to consider is the type of apparatus chosen. The USP type I (basket) and II (paddle) apparatus remain the most popular equipment for dissolution testing in drug development, partly due to familiarity and availability of the equipment, but also due to how robust they have proven at providing useful insights into dosage form performance. In particular, they have proven useful in quality control when used under sink conditions to demonstrate complete release of hydrophilic molecules (Kostewicz et al., 2014b). However, their use has also extended from the QC process to that of drug development, particularly when coupled with biorelevant media at physiological volumes. The paddle apparatus has been frequently used for BCS class II drugs in IR dosage forms in conjunction with

42

biorelevant media to predict *in vivo* performance and identify formulation and food effects. The use of type III (reciprocating cylinder) compendial apparatus in development has been largely restricted to modified release dosage forms, with relatively few published examples (Fotaki and Vertzoni, 2010, Klein et al., 2013). Type IV (flow through cell) is increasing in popularity in drug development, and in IVIVC generation, particularly for controlled release formulations (McCarthy et al., 2017, Sunesen et al., 2005, Jantratid et al., 2009).

The vast majority of dissolution tests continue to be carried out in conventional, USP I and II compendial apparatus, with a single static medium, at a constant pH, volume and which may or may not include an absorption sink. These conditions do not adequately mimic the *in vivo* situation, where gastrointestinal motility exposes the formulation to a complex, rapidly evolving luminal environment (Kostewicz et al., 2014b). In order to better reflect the *in vivo* environment, more biorelevant dissolution methods which mimic the dynamic gastrointestinal environment have been developed.

The simplest such method is the two-compartment transfer model first designed by Kostewicz *et al.* (Kostewicz et al., 2004). This experimental set-up consists of two USP type II vessels side by side and simulates transfer from the stomach to the intestine by first placing a drug solution in a simulated gastric fluid compartment which is transferred into the simulated intestinal compartment at a constant rate, reflective of gastric emptying rate, *via* a peristaltic pump. Such a set-up is useful in both modelling the impact of gastric emptying and can be used in the assessment of

supersaturation and precipitation. While the type IV apparatus can facilitate media changing in an "open" setup, such set-ups are not commonly used (Fotaki, 2011).

Even with the development of such transfer models, the limitations of a static dissolution vessels in any of the compendial apparatus for simulating the dynamic in vivo processes of gastric emptying, absorption, changes in pH and fluid composition and volume and intestinal transit are well reported. This has led to the development of "GI tract in lab" systems, which are more complex systems incorporating numerous compartments and various levels of control on processes such as gastric emptying. Having originated from research in the nutritional sector, there are several examples of these apparatus being used to assess formulation performance and the impact of food on bioavailability. Systems such as the TIM-1 and TIM-2 systems (Brouwers et al., 2011, Lyng et al., 2016) and the dynamic gastric model (DGM) (Thuenemann et al., 2015, Mason et al., 2016, Chessa et al., 2014) are dynamic multi-compartmental simulators intended to mimic the main mechanical and chemical functions of the gastrointestinal tract, as well as the effect of the presence or absence of absorption sinks. There has been relative success with these apparatus, however, their scant availability, prohibitive cost and lack of systematic validation has limited their widespread use in industry. While such methods hold some promise for improved *in vivo* predictions, evaluation and validation remain anecdotal. More widespread utilisation of these methods has also likely been limited due to the relative success of integrating simple solubility and dissolution measurements with *in vivo* pharmacokinetic data through the use of PBPK models, which is further discussed below.

Permeability and absorption

While dissolution assessment can provide a useful prediction of dissolution performance *in vivo*, one of the major challenges with biorelevant dissolution methods can be the absence of an absorption sink. A significant drawback of conventional dissolution methods is the inability to mimic the *in vivo* absorption of drug in the static *in vitro* environment in a satisfactory manner. As dissolved or solubilised drug remains present in dissolution apparatus, this can create an inhibitory effect on dissolution or even stimulate precipitation. This is particularly likely for poorly soluble, lipophilic compounds where rapid absorption is likely *in vivo* and may be compensatory for poor dissolution (Butler and Dressman, 2010). Simply ignoring absorption during dissolution assessment may compromise the biorelevance and predictive capacity of the test (Takano et al., 2012). Traditional approaches to overcome this have focused on the utility of synthetic surfactants to generate sink conditions, or simply through predicting drug flux and fraction absorbed, based on combining measured concentrations with drug permeability (Kostewicz et al., 2014b). The use of alternative dissolution media, where these compounds will not demonstrate poor solubility and therefore sink conditions will be maintained, such as those traditionally used for quality control, risks the loss of biorelevance. For this reason, a number of approaches have been investigated in combination with traditional dissolution methodology to mimic the absorptive *in vivo* process.

Alternative methods have been developed, where an absorption step is taken into account, either in the form of a biphasic dissolution set-up, where the absorptive sink is provided by the use of a non-miscible organic solvent layer (Shi et al., 2010, Pestieau and Evrard, 2017), or the combined use of a dissolution/permeation (D/P) apparatus (Kataoka et al., 2012, Buch et al., 2009, Miyaji et al., 2016). Such D/P apparatus may use either cellular based absorption assays, most often a Caco-2 monolayer, or indeed may use tissue-based techniques, such as *in situ* perfusion studies of intestinal segments, most likely rat intestine, or using Ussing chamber mounted tissue. These systems have also been adapted for use with biorelevant media (Markopoulos et al., 2014, Wuyts et al., 2015a, Wuyts et al., 2015b, Stappaerts et al., 2014). By coupling the dissolution and permeation process, the complex interplay of dissolution and permeation can be more accurately assessed. These systems vary significantly in set-up, operation and biorelevance, and while integrating absorption into *in vitro* dissolution assessment is critical for accurate prediction of performance of certain bioenabling formulations, the benefit of some of these approaches remains unclear and their validation, too, is often anecdotal.

With the proliferation of *in vitro* tools to predict *in vivo* performance, there is now a wide array of tools of varying levels of complexity available to forecast formulation behaviour. However, there remains significant gaps in our ability to model all the physiological determinants of absorption. For this reason, there has been significant development of *in silico* models which combine data from these newly developed, biorelevant screening tools with physiological measurements to model and predict *in vivo* performance, as described here.

46

In silico methods

Prediction of human pharmacokinetics generally relies on interpretation and extrapolation from *in vitro* and preclinical *in vivo* data (Suenderhauf and Parrott, 2013). There has been significant development in computational approaches to predict human pharmacokinetics, ranging from classical computational absorption simulation based on compartmental PK through to more complex physiologically based pharmacokinetic (PBPK) models, which can integrate data from both these sources to give an estimate of human PK (Dressman et al., 2011). Despite the improvements in the biorelevance of *in vitro* screening over the last 25 years, routine dissolution screening as currently implemented cannot accurately model all the dynamic *in vivo* processes involved in drug absorption and meaningful IVIVC for immediate release, bioenabling formulations remains elusive. Gastrointestinal transit, permeability measurement, dynamic changes in pH, luminal physicochemical properties and first pass extraction, among other variables, are factors which can influence drug and formulation performance but are not measured in commonly used *in vitro* tests. These factors play a crucial role in determining the rate and extent of bioavailability from immediate release formulations, and the inability to routinely calculate these variables *in vitro* limits the capacity to generate direct IVIVC.

As an alternative approach to the increasing complexity of *in vitro* techniques or the proliferation of *in vivo* studies, integration of *in vitro* analysis with *in vivo* pharmacokinetic data, through the use physiologically based pharmacokinetic (PBPK) models, not only allows estimation of the overall rate and extent of bioavailability, but also enables prediction of the overall disposition and elimination process,

allowing prediction of a simulated drug plasma profile (Shono et al., 2009, Flanagan et al., 2016, Dressman et al., 2011, Shono et al., 2010).

PBPK models are mathematical models that integrate drug physicochemical and *in vitro* data with *in vivo* physiological data to simulate pharmacokinetic profiles and systemic tissue exposures, allowing their prediction from preclinical *in vitro* and *in vivo* data (Jones et al., 2015). The distinguishing feature of PBPK models, relative to empirical computational models, is the application of prior physiological knowledge in the mechanistic mapping of model compartments and in the processes that determine absorption (Suenderhauf and Parrott, 2013, Kostewicz et al., 2014a). This physiological knowledge, incorporating parameters such as gastrointestinal transit, pH and luminal volume, is combined with physiochemical measurements, for example, dissociation constants and partition coefficients, and *in vitro* measurements, such as solubility and dissolution rates and enzymatic degradation kinetics, into the PBPK model to provide a simulated PK profile (Dressman et al., 2011, Suenderhauf and Parrott, 2013). PBPK models allow predictions of drug disposition based on a series of mass-balance equations, which incorporate physiological, physiochemical and *in vitro* data within an *in silico* model. They offer a significant advantage in enabling integration of all collected data into a single model so that the relative importance of each can be assessed and CQAs identified (Hansmann et al., 2016). Numerous commercial PBPK software systems are available, most notably Simcyp®, GastroPlus® and PK-Sim®, while there is also widespread use of user-built models, built using packages such as MATLAB, Berkeley Madonna, MoBi, STELLA or acslX® (Kostewicz et al., 2014a). While the structure and functionality of

these programmes can vary greatly, a common feature is the mathematical modelling of all relevant processes to the GI absorption of drugs, including disintegration, solubility and dissolution, precipitation, uptake and efflux, first pass metabolism and gastrointestinal transit to predict the rate and extent of drug absorption, while also providing a mechanistic understanding of interplay of these various factors. Such a tool is particularly useful, not only for linking *in vitro* and *in vivo* data, but also allowing a virtual, mechanistic exploration of the critical factors and parameter values affecting absorption, potentially identifying quantitative relationships between drug and formulation factors and *in vivo* outcomes (Kostewicz et al., 2014a, Hansmann et al., 2016).

Numerous PBPK models have been successfully utilised to identify the key issues in the drug development. To date these models have been particularly successful with regard to late stage formulation bridging, assessing the potential for drug-drug interactions, examining effects in special populations, such as those displaying enzymatic polymorphism and in disease states, such as hepatic impairment and achlorhydria (Wagner et al., 2012, Kesisoglou, 2014, Jones et al., 2015).

The successful prediction of plasma profiles relies on the ability of *in vitro* models to accurately simulate true *in vivo* values. As the drug development process proceeds, more accurate and biorelevant data is generated, particularly in the late preclinical and early clinical stages. Continual refinement of PBPK models with this data will result in a more predictive model, capable of accurately modelling *in vivo* performance and identifying CQAs. Appropriate use of these models and interpretation of results from these simulated studies has the potential to reduce

both the time and cost of drug development (Lave et al., 2007). Recent studies have demonstrated a reasonable reliability of PBPK models to simulate post-absorptive events. However, true prospective "bottom up" prediction of complete oral pharmacokinetic profiles for a drug/ formulation remains a pipedream (Hansmann et al., 2016). Currently, the most practical use of PBPK models involves "middle out" approaches, with a 'learn and confirm' paradigm adapted, through using observed *in vivo* data to refine and optimise existing PBPK models (Kostewicz et al., 2014a).

The future development of improved PBPK models relies on the continuing development of biorelevant *in vitro* screening methods and increased ability to link these bench-top tests to clinical performance, while also continually validating these approaches. Measurement and prediction of permeability related parameters is of particular interest (Hansmann et al., 2016). To date, the lack of systematic validation limits the widespread implementation PBPK models. While regulatory agencies are increasingly receptive of *in silico* modelled data as part of submission dossiers, with FDA recently publishing guidelines for submission of PBPK derived data, there remains significant scope for increased acceptance of validated PBPK data (FDA, 2016). There is a strong need for better understanding of pharmaceutics/biopharmaceutics factors to improve predictions of GI drug absorption using these *in silico* tools (Poulin et al., 2011). Continuous improvement of the predictive capacity of these models will further enhance the regulatory acceptance (Jones et al., 2015).

Pre-clinical animal studies

While the use of biorelevant *in vitro* and *in silico* approaches for formulation assessment, with regard to predicting bioavailability and disposition, have become increasingly refined, there remains a significant knowledge gap on the interplay of the various biopharmaceutical properties, namely drug physicochemical characteristics, formulation properties and gastrointestinal physiology, on *in vivo* drug absorption and pre-clinical *in vivo* assessment remains the mainstay of diagnosing biopharmaceutical performance (Grignard et al., 2016). Despite obvious and well publicized differences in both physiology and in drug/formulation performance between species, pre-clinical animal models are still widely regarded as the most accurate predictor of bioavailability in humans.

The conventional drug product development process involves initial *in vitro* screening followed preclinical testing before proceeding to clinical evaluation in humans. There are two key stages where improvements can be made with regard to our understanding of the biopharmaceutical prediction of drug and formulation performance. Firstly, the link between the *in vitro* testing and the preclinical *in vivo* performance, there is a need for an improved biorelevance of *in vitro* screening techniques such that they become more predictive of pre-clinical performance, either through direct IVIVC or in conjunction with PBPK modelling, and *in vivo* testing becomes truly confirmatory rather than investigatory. Secondly, there is a need to validate the reliability of preclinical models to predict performance in humans so that clinical studies become confirmatory of preclinical investigations. Improving both of these aspects of preclinical development involves the selection of both the

appropriate *in vitro* and *in silico* screening tools, along with prudent selection of a pre-clinical species for *in vivo* assessment

There remain considerable gaps in our knowledge regarding the appropriate animal models for the assessment of oral bioavailability in humans and often animal studies yield species specific differences and pose the question of which animal species is most representative for humans (Lennernas et al., 2014, Henze et al., 2018b, Sjogren et al., 2014). These pre-clinical studies often involve a range of species, most commonly rats, dogs and non-human primates. While these models have proven useful in drug development, there remains significant drawbacks to each. Such limitations can include differences in anatomy and physiology of the GI tract and difficulty in study setup, such as designing a dosage regimen, amount of water or food, the type of meal, chewing patterns and route of administration, where these factors do not mimic the intended use of the dosage form in humans. In particular, the relatively small size of rats means that the potential for dosing intact dosage forms is limited while there is significant deviation the physiology of the GIT, particularly with regard to the secretion of bile (Sjogren et al., 2014, Davies and Morris, 1993). Dogs are the most widely utilised large animal model for prediction of human bioavailability, however, differences in gastrointestinal anatomy and physiology, principally their relatively short small intestine, high gastric pH and differences in intestinal and hepatic metabolism patterns limits their utility in certain instances (de Zwart, 1999, Dressman, 1986). Non-human primates are widely regarded as the most representative organisms when it comes to predicting bioavailability in humans, however divergences still remain in metabolic pathways,

while prohibitive cost and ethical implications prevent their more widespread use (Hatton et al., 2015, Sjogren et al., 2014). Thus, while there is a well-established history of using these pre-clinical models, there is also significant limitations of these animal models, with regard to their ability to predict human *in vivo* performance (Sjogren et al., 2014, Hatton et al., 2015, Musther et al., 2014). Nevertheless, pharmaceutical development relies on these studies, since animals represent complete organisms necessary to replicate the complex interplay of drug dissolution, permeation and metabolism. Recognising the limitations of a "one size fits all" approach in the context of animal modelling, and the absence of one ideal species that mimics closely human GI physiology and function, the most reliable approach appears to be the utilisation of numerous different animal models to model the various aspects of human pharmacokinetics (Hatton et al., 2015).

In order to streamline pre-clinical development and reduce repeated testing in various animal models it is necessary to get a better understanding which particular animal model is suitable for a specific drug candidate and how predictive of humans it will be (Henze et al., 2018b). While there has been extensive discussion in the literature on the use of dog and non-human primate models to predict oral bioavailability in humans, in relation to the pig models there are significant gaps in our understanding.

The pig in pre-clinical drug development

The pig presents numerous advantages in the assessment of pre-clinical formulations, particularly with regard to the anatomical and physiological similarities in the gastrointestinal tract of pigs and humans and can be considered a translational model (Hatton et al., 2015, Swindle and Smith, 1998, Puccinelli et al., 2011, Suenderhauf and Parrott, 2013, Sjogren et al., 2014). In this regard, the use of the pig model in pre-clinical assessment has expanded significantly in recent years (Colleton et al., 2016). Pigs have, therefore, become increasingly popular as an alternative species in drug development (Forster et al., 2010, Helke and Swindle, 2013, Bode et al., 2010, Ganderup et al., 2012). However, the potential use of pigs as an *in vivo* model for drug formulation research and development remains relatively unclear and requires further exploration. This is also complicated by the fact that there are several breeds of domestic and minipigs used in research studies, including the domestic landrace pig and the Göttingen and Yucatan minipigs, which may have considerably different characteristics and traits, but which are most often classified and summarised together (Henze et al., 2018b, Sjogren et al., 2014). To understand in which circumstances pigs should be considered, a clear understanding of the conditions in the GI tract is essential.

Henze *et al.* (2018b) have recently summarised the use of the pig model in preclinical studies, reviewing similarities and differences between porcine and human gastrointestinal structure, function and physiology as part of the work. While there are many similarities in the gastrointestinal structure and physiology between pigs and humans, there are also significant differences. Similarities are particularly

evident with regard to the gastrointestinal pH profile and intestinal morphology and relative length of gastrointestinal sections. However, significant variations exist particularly with regard to gastrointestinal transit and metabolising enzymes (Henze et al., 2018b). These similarities are differences in porcine and human gastrointestinal are described in greater detail below.

Gastrointestinal anatomy

The internal physiology of humans and pigs is quite similar to that of humans, with the presence of similar thoracic and abdominal organs. With regard to the GIT, most obvious similarities between pig and human is that both are monogastric omnivores, with glandular stomachs where acid secretion occurs as a function of exogenous and endogenous stimuli, such as food intake and gastric volume, while bile secretion is also stimulated by food intake (Sjogren et al., 2014, Hatton et al., 2015). While the overall size of the pig GIT is larger than that of humans, with a greater stomach capacity (pigs; 8L: humans; 1-1.6L) and intestinal length (pigs; 24cm/kg: humans; 14cm/kg), the internal diameter, at 2.5 – 3.5cm, and the relative length of the major intestinal structures, namely the stomach, small intestine and large intestine, are quite similar (Henze et al., 2018b, Merchant et al., 2011, Suenderhauf and Parrott, 2013, Kararli, 1995, Hatton et al., 2015).

With a significantly longer small intestine, the smooth luminal surface area available for absorption in pigs is significantly greater than that in humans, and this large surface area is thought to facilitate high levels of absorption (Hatton et al., 2015). However, DeSesso and Williams have suggested that their total surface area is more comparable when taking into account the increased surface area generated by the

plicae, villi and micro-villi of the apical brush border (DeSesso and Williams, 2008) A notable anatomical difference between pigs and humans is the structure of the large intestine, where the pig cecum, ascending and transverse colon and the proximal portion of the descending colon are arranged in a series coils, known as the spiral colon, which may be relevant for colonic targeted drug delivery (Henze et al., 2018b).

Gastrontesinal fluid pH, volume and characterisation

The gastric pH in fasted pigs and humans is broadly similar, though pig gastric pH is somewhat variable (1.2–4.0) relative to humans (1.0 – 3.5), with an indication of regional pH variation within the porcine stomach (Hossain et al., 1990, Oberle and Das, 1994, Sjogren et al., 2014). An overview of the pH along the length of the GI tract of both humans and Landrace pigs is presented in Table 1-3, and this demonstrates a similar increase in the pH along the length of the small intestine, and slight reduction in the colon in both species. Similar trends were noted in the pH profiles in both the fasted and fed states in both pigs and humans. The effect of food in buffering the gastric pH, while also resulting in a slight reduction in small intestinal pH through a gastric emptying effect, is seen in both species. This is one distinct advantage of the porcine model compared to the canine model and is particularly important with regard to predicting the *in vivo* performance of weakly acidic and basic compounds (Sjogren et al., 2014, Henze et al., 2018b).

Table 1-3 Comparison of pH in the gastrointestinal tract of humans (and landrace pigs (adapted from (Henze et al., 2018b)

Segment	Human		Landrace Pig	
	Fasted pH [a, b]	Fed pH [a, b]	Fasted pH [b, c]	Fed pH[d]
Stomach	1.0-3.5	3.0-6.0	1.2-4.0	4.4
Duodenum	6.0-7.0	5.0-5.5	6.7	6.1-6.5
Jejnum	6.0-7.7	5.0-6.5	6.8	6.3-6.6
Ileum	6.5-8.0	6.5-8.0	6.9	6.5-6.7
Colon	5.5-8.0	6.0-7.5	6.1-6.6	6.5-6.6

[A] *(Abuhelwa et al., 2016a)*
[B] *(Kararli, 1995)*
[C] *(Hossain et al., 1990)*
[D] *(Merchant et al., 2011)*

While pH profiles are quite similar, Merchant *et al.* have compared to the gastrointestinal fluid volumes of pigs and humans. Compared to pigs (~1545g, 20 g/kg body weight, or 0.65 g/cm gut length) lower fluid volumes are reported in the human gut (~517 g, 8.2 g/kg body weight, or 0.58 g/cm gut length) (Merchant et al., 2011, Hatton et al., 2015). Most of this fluid volume observed in humans is actually in the bound state, as indicated by the much smaller amounts of free water (54 ± 41 mL in small intestine and 11 ± 26 mL in colon), which is mostly present in fluid pockets along the length of the intestine (Schiller et al., 2005). Conversely, it has been suggested from post-mortem studies that fluid present in pigs is more 'free-flowing', and that this may enhance the suitability of pigs in assessing the bioavailability of dissolution rate limited drugs (Merchant et al., 2011, Hatton et al., 2015).

Merchant *et al.* have also compared the buffer capacity, osmolality and surface tension of gastrointestinal aspirates between pigs and humans. Buffer capacity of human duodenal aspirates was similar in pigs and humans, though ileal and jejunal aspirates from pigs had appreciably higher buffer capacity than those of humans. While gastric osmolality was higher in humans than pigs, this is greatly influenced by the composition of an administered meal, and that observed in the small intestine is

similar in both species. Surface tension, meanwhile, was significantly lower in human aspirates compared to those of pigs (Merchant et al., 2015).

An area where data remains relatively sparse is in the bile salt concentrations in both fasted and fed state gastrointestinal fluid in pigs (Henze et al., 2018b). Significant work has contributed to the profiling of bile salts in other porcine physiological fluids, as well as bile flow rates, however, gastrointestinal concentrations have not been quantified (Bergman et al., 2009, Juste et al., 1983, Alvaro et al., 1986, Scanff et al., 1997). This measure is of critical importance in assessing the solubilising capacity of porcine gastrointestinal fluid for PWSD (Holm et al., 2013b).

Gastrointestinal transit

Pigs appear to demonstrate slow and variable gastric emptying, and this is an important species difference to human with high potential implications for the *in vivo* investigations of bioenabling formulations, while also having particular implications for investigating food effects in the pig model (Davis et al., 2001, Oberle and Das, 1994, Patterson et al., 2008, Hossain et al., 1990). In fact, Henze *et al.* have recently failed to demonstrate any significant variation in gastric emptying in fed or fasted minipigs, even where a prokinetic (metoclopramide) agent was administered (Henze et al., 2018a). This is a finding which has also been demonstrated elsewhere previously, and this may be a factor in designing fasting regimens for fasted state bioavailability studies in pigs (Suenderhauf et al., 2014, Christiansen et al., 2015).

The gastric emptying rate in pigs is considered to be longer and more variable than in humans, where a recently assessed meta-mean of 1.37 hours in the fasted state, which increases in the fed state as a function of caloric content and meal volume, has

been reported (Abuhelwa et al., 2016b). The values reported for gastric emptying rate in pigs vary significantly, with many potential sources of such variation including the distinctive bimodal and incomplete gastric emptying, the presence of a unique muscular out-pouching which can lead to food retention or, indeed, methodological differences in how gastric emptying is assessed (Hatton et al., 2015, Henze et al., 2018b). Values ranging from 1.1-2.2 hours for liquids and pellets and 1.5 – 6 hours for tablets, to 6 to 24 hours have been reported for gastric emptying rate in pigs (Oberle and Das, 1994, Davis et al., 2001). These inconsistent and variable values have been the focus of renewed attention recently, with Christiansen *et al.* and Suenderhauf and co-workers investigating the effects of various dietary regimens and pharmacological interventions on gastric emptying in an attempt to improve the predictive capacity of porcine models of oral absorption, with a particular focus on mini-pigs (Suenderhauf and Parrott, 2013, Suenderhauf et al., 2014, Christiansen et al., 2015). One of the aims of the current thesis is to focus on further characterisation of gastric emptying in Landrace pigs, with a particular emphasis on the effects of dietary regimens on gastric emptying.

Intestinal transit times, in contrast to gastric transit times, are more comparable to that in humans, while also appearing to be relatively consistent at 3–4 hours in pig compared to 2–4 hours in humans (Davis et al., 2001, Gardner et al., 1996, Suenderhauf et al., 2014). In summary, the gastric emptying rate appears to be significantly longer and more variable in pigs relative to humans, while the small intestinal transit time appears both more conserved and reflective of that in humans.

Intestinal metabolism and transporters

Another area where there is uncertainty with regard to the predictive capacity of the porcine model is with regard to intestinal permeability, enzymatic metabolism, both intestinal and hepatic, and intestinal uptake and efflux transporters. With regard to permeability, intestinal enzymes and transporters, the principal drawback is a lack of data and clarity, where homology has not been widely characterised and mechanistic studies are required to determine comparability. While Westerhout *et al.* have used *ex vivo* intestinal tissue to compare porcine and human P_{app}, and demonstrated its superiority to Caco-2 cultures, the number of such studies is low and needs further validation (Westerhout et al., 2014). Vaessen *et al.* have recently investigated expression levels of transporters and enzymes along the pig intestinal tract, demonstrating relative comparability, indicating further promise. However, expression levels of specific transporters, most notably breast cancer resistance protein (BCRP), multidrug resistance proteins (MRP) 1 and 3 and organic anion transporter protein (OATP) 4A1 differ significantly between the species, indicating there may be limitations in drugs subject to significant levels of intestinal efflux in the pig model (Vaessen et al., 2017).

Hepatic enzyme homology is also an area of concern when choosing the pig as a potential animal model. While CYP 1A1, 1A2, 2B, 2E1 and 3A (3A4 in particular) display good homology between pigs and humans, this is not the case with other CYP families (Puccinelli et al., 2011, Anzenbacherova et al., 2005). In particular there appears to be less comparability of CYP 2C and 2D families (Helke and Swindle, 2013, Puccinelli et al., 2011, Anzenbacher et al., 1998, Thörn et al., 2011). Further

investigations *in vitro*, *ex vivo* and *in vivo* for the characterization of the intestinal permeability, metabolism (especially that mediated by CYP2C and CYP2D enzymes) and transporters are required for optimum application of the pig as a preclinical model (Sjogren et al., 2014).

Summary

Overall, while the pig model displays relative similarities to the GI conditions of humans anatomical and physiological perspective, there are also significant limitations to its utility to predict human bioavailability of oral dosage forms. While some of this variability is due to inherent differences between porcine and human physiology, it is also at least partially due to remaining gaps in our knowledge regarding the pig model, particularly regarding gastric emptying and fasting protocols to ensure a true 'fasted' state, the food effect and the effects of metabolic enzymes. There is an overall need to harness knowledge from a wider range of drug molecules to assess the utility of the pig model's predictive capacity. There is also an opportunity to improve the link between *in vitro* screening and *in vivo* testing in preclinical animals through developing a deeper mechanistic understanding of the pig model.

To this end, the current thesis focuses on further characterisation of the pig model in pre-clinical formulation assessment. Particular emphasis is placed on the ability of the pig to screen bioenabling formulation performance, to act as a model of food effect and how representative the pig model is of human bioavailability. The effect of various fasting protocols to ensure complete fasting is assessed. The ability of biorelevant *in vitro* and *in silico* tools to forecast pre-clinical *in vivo* performance in pigs is also evaluated.

61

Summary

Methods of characterizing drug and formulation performance have evolved substantially since they were first introduced as quality control tools. As a greater understanding of the biopharmaceutical aspects of oral drug delivery has been gained, the biorelevance of the screening tools has consequently increased significantly, as has the ability to predict *in vivo* performance. There remains a dichotomy in pre-clinical evaluation, where there is a desire for a screening tool which is "as simple as possible, but as complex as necessary" to predict *in vivo* performance. In practice it will not be possible to capture all the aspects of drug absorption in one simple to use, routine *in vitro* test method, rather a suite of tools is required. This toolkit is likely to be diverse and with varying levels of complexity, sometimes focusing on a single aspect of the *in vivo* environment likely to be critical to a specific drug product, such as lipolysis of LBF, and on other occasions needing to be capable of mimicking multiple aspects of importance to *in vivo* performance, such as the use of GI tract in lab apparatus. These tests will also provide crucial inputs for PBPK modelling, allowing both prediction of *in vivo* pharmacokinetics and mechanistic investigation of the critical factors affecting *in vivo* performance. To improve the biorelevance and predictive capacity of these approaches there needs to be an increase in attempts of systematic validation of these approaches. This involves both the assessment of the predictive capacity of these *in vitro* tools of *in vivo* performance, through generating IVIVC either directly or through *in silico* modelling, while also increasing the number of *in vivo* data at our disposal to allow validation of these tools. The attempts to address these issues are among the primary aims of the current thesis.

Model compounds

In order to assess the utility of the approaches described above, a range of model

compounds is required. Two PWSD were used in this thesis and are described here.
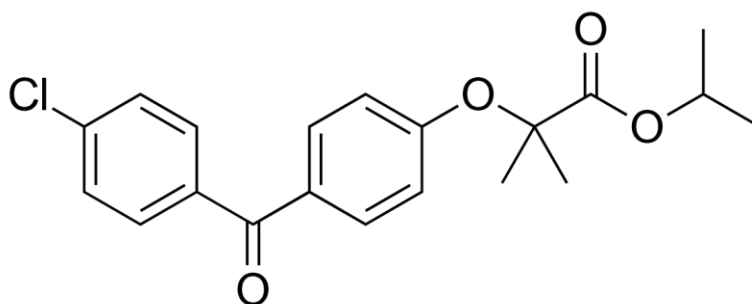
Fenofibrate

Figure 1-9 Chemical structure of fenofibrate

Fenofibrate (figure 1-9) is an orally active fibric acid derivative used in the treatment

of hypercholesterolemia (Miller and Spence, 1998). As a neutral molecule with a high

dose: solubility ratio, fenofibrate is a model poorly soluble drug and is often used as

a test sub-stance to evaluate novel bioenhancing strategies. Pharmacokinetic

evaluation of fenofibrate is based on quantifying its major active metabolite,

fenofibric acid, which it is rapidly and completely converted to by gut wall esterases

(Miller and Spence, 1998). Fenofibrate has been shown to be amenable to re-

formulation in dissolution enhancing preparations, with subsequent improvements

in bioavailability allowing dose reduction and food-independent dosing of these

novel formulations (Ling et al., 2013). Such formulations include micronised,

nanosized and lipid-based formulations, demonstrating that approaches to enhance

solubility and dissolution of fenofibrate can improve its bioavailability, both in

preclinical studies and in clinical use (Guichard et al., 2000, Sauron et al., 2006, Fei et

al., 2013). The Lipantil Micro® formulation, a micronised product, displays food

63

dependent bioavailability, and therefore requires administration with food. A re-formulated product, Lipantil® Supra was developed using NanoCrystal® technology, to overcome this limitation and allows food independent administration and dose reduction (Sauron et al., 2006, Guichard et al., 2000, Junghanns and Muller, 2008).
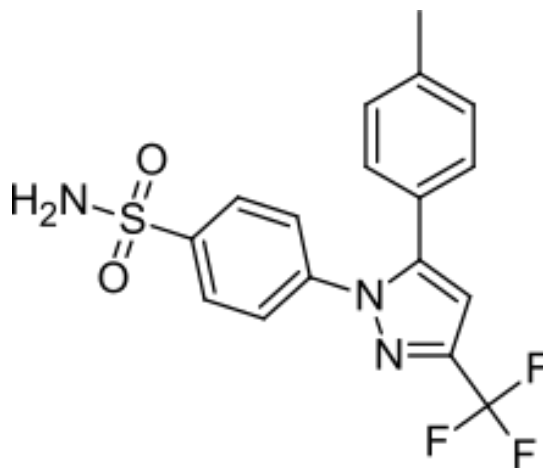
Celecoxib

Figure 1-10 Chemical structure of celecoxib

Celecoxib (figure 1-10) is a non-steroidal anti-inflammatory drug (NSAID) which exerts its pharmacological action by selective inhibition of the cyclooxygenase-2 (COX-2) isozyme. It is widely used in the treatment of osteo- and rheumatoid arthritis and ankylosing spondylitis (Shi and Klotz, 2008, Davies et al., 2000). Celecoxib is highly lipophilic and very poorly water soluble, with an approximate aqueous solubility of 1μg/ml, but demonstrates good permeability and is classed as a BCS class II compound with dissolution/solubility limited oral absorption (Guzman et al., 2007, Paulson et al., 2001, Laine et al., 2016). As a result, the marketed Celebrex™ formulation was designed to maximise dissolution, with particle size identified as a critical quality attribute (CQA) during the regulatory process. The commercial preparation has a D_{90} of below 25μm and the addition of sodium lauryl sulphate as a

wetting agent, with the function of improving dissolution of the API *in vivo* (Laine et al., 2016, FDA, 1998). As a BCS class II compound, a significant positive food effect is anticipated for celecoxib. However, while a significant increase in C_{max} (1.9 fold increase) is observed in the fed state, mediated by increases in post-prandial solubilisation, there is only a modest increase in overall bioavailability (approximately 1.1 to 1.3 fold), allowing Celebrex™ to be dosed independent of prandial state (Paulson et al., 2001, Pfizer Inc., 2000, Lyng et al., 2016).

Celecoxib bioavailability has previously been shown to be highly variable, with a coefficient of variation (CoV) in AUC shown to vary between 40-78% in fasted humans. Differences in metabolism mediated by enzymatic polymorphism have been shown to have a significant effect on celecoxib pharmacokinetics and exposure. CYP2C9 is the primary enzyme involved in celecoxib metabolism (Paulson et al., 1999, Gong et al., 2012) and genetic variation in this enzyme reduces clearance and can more than double celecoxib exposure, with drug label warnings expressing caution in use of celecoxib in patients known to be poor 2C9 metabolisers owing to the risk of observing abnormally high plasma levels of celecoxib (Kirchheiner et al., 2003, Tang et al., 2001, Pfizer Inc., 2000). In such cases, using celecoxib at half the recommended lowest dose is advised.

Thesis objectives

In light of the factors described here, the specific objectives of this thesis are, therefore, to assess novel bioenabling formulations and new *in vitro* and *in silico* tools to predict their *in vivo* performance in pigs as a means to improve efficiency in formulation development. The current thesis aims to shed new insights on the processes involved in drug product development. Specifically, we have assessed the utility of the pig as a pre-clinical animal model with regard to the assessment of bioenabling approaches, using dissolution enhancing formulations and by concomitantly administering dosage forms with food. By utilising the current approach, the principle aims of the current thesis were;

1. To assess the ability of novel bioenabling formulations for the enhancement of oral bioavailability of poorly water soluble drugs, with a specific focus on eliminating food-effects

2. To investigate the utility of the pig to act as a model species for the assessment of these bioenabling formulations

3. To assess the ability of biorelevant screening approaches, in conjunction with *in silico* approaches of varying levels of complexity to predict *in vivo* performance

4. To investigate the pig as a potential model for food effect bioavailability as an alternative bioenabling approach, particularly with regard to the physiological conditions in the pig in the fasted and fed state.

The approaches involved in assessing these aims are described in detail in the proceeding chapters, and an overall, general discussion of the findings and how they relate to the wider literature is also provided.

Chapter 2 : Mesoporous silica-based dosage forms improve bioavailability of poorly soluble drugs in pigs: case example fenofibrate

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Abstract

Objectives: Mesoporous silicas (SLC) have demonstrated considerable potential to improve bioavailability of poorly soluble drugs by facilitating rapid dissolution and generating supersaturation. The addition of certain polymers can further enhance the dissolution of these formulations by preventing drug precipitation. This study uses fenofibrate as a model drug to investigate the performance of an SLC-based formulation, delivered with hydroxypropyl methylcellulose acetate succinate (HPMCAS) as a precipitation inhibitor, in pigs. The ability of biorelevant dissolution testing to predict the *in vivo* performance was also assessed.

Key findings: Fenofibrate-loaded mesoporous silica (FF-SLC), together with HPMCAS, displayed significant improvements in biorelevant dissolution tests relative to a reference formulation consisting of a physical mixture of crystalline fenofibrate with HPMCAS. *In vivo* assessment in fasted pigs demonstrated bioavailabilities of 86.69 ± 35.37% with combination of FF-SLC and HPMCAS in capsule form and 75.47 ± 14.58% as a suspension, compared to 19.92 ± 9.89% with the reference formulation. A positive correlation was identified between bioavailability and dissolution efficiency.

Conclusions: The substantial improvements in bioavailability of fenofibrate from the SLC-based formulations confirm the ability of this formulation strategy to overcome the dissolution and solubility limitations, further raising the prospects of a future commercially available SLC-based formulation.

Introduction

The poor aqueous solubility and resulting slow and/or incomplete *in vivo* dissolution of poorly soluble drugs often limits their bioavailability after oral administration. Adsorption onto mesoporous silica has demonstrated considerable potential in enhancing the oral bioavailability of poorly soluble drugs (Van Speybroeck et al., 2011, Vialpando et al., 2011). These bioenabling formulations improve absorption of such drugs through the generation of supersaturation, a metastable state in which dissolved drug concentration exceeds the equilibrium solubility in the medium (Brouwers et al., 2009, McCarthy et al., 2016).

Adsorption of drug onto a mesoporous silica carrier has a number of advantages for enhancing drug dissolution. The ordered mesoporous structure and high porosity of silica allows high drug loads to be adsorbed (Vialpando et al., 2011). Drug molecules adsorb to the silica surface in a noncovalent manner, principally through electrostatic interaction, hydrogen bonding or van der Waal's forces, which are easily broken on exposure of the formulation to an aqueous environment, enabling release of drug in its molecular form and facilitating absorption. The drug can exist in an amorphous or molecularly dispersed state on the silica surface, thus displaying higher apparent solubility and dissolution rate compared to the crystalline substance (Hancock and Zografi, 1997). Although amorphous materials can display thermal instability, adsorption onto the mesoporous silica has proven effective in stabilising amorphous systems, and the long-term stability of these formulations has been repeatedly demonstrated (McCarthy et al., 2016, Van Speybroeck et al., 2009). By controlling pore size and volume as well as the surface chemistry, the formulation can be tailored

69

for each drug molecule with regard to its physicochemical characteristics. The optimised preparation can maximise drug loading and facilitate long-term stability via inhibition of recrystallization within the limited pore space (Salonen et al., 2008).

The favourable dissolution and solubility behaviour associated with these formulations and their ability to generate supersaturation has been described as the 'spring' that enhances absorption and bioavailability (Brewster et al., 2008). However, a potential risk of such strategies is the possibility of precipitation from the supersaturated solution that is formed during drug release. For this reason, bioenabling drug delivery systems often also include a 'parachute' which aims to stabilise and prolong the supersaturated state (Guzman et al., 2007). One method to create a 'parachute' is the inclusion of a polymeric precipitation inhibitor as a functional excipient to slow the rate of precipitation (Van Speybroeck et al., 2010b, Warren et al., 2010, Laine et al., 2016). The mechanism by which these excipients prevent crystallisation is by interacting with drug molecules and thus precluding nucleation, which is a pre-requisite to precipitation. Examples of polymeric precipitation inhibitors include polyvinylpyrrolidine (PVP), hydroxypropyl methylcellulose (HPMC), hydroxypropyl-methyl cellulose acetate succinate (HPMCAS) and copovidone (PVPVA) (Dressman et al., 2016).

Fenofibrate is an orally active fibric acid derivative used in the treatment of hypercholesterolemia (Miller and Spence, 1998). As a neutral molecule with a high dose: solubility ratio, fenofibrate is a model poorly soluble drug and is often used as a test substance to evaluate novel bioenhancing strategies. Fenofibrate has been shown to be amenable to re-formulation in dissolution enhancing preparations, with

subsequent improvements in bioavailability allowing dose reduction and food-independent dosing of these novel formulations (Ling et al., 2013). Such formulations include micronised, nanosized and lipid-based formulations, demonstrating that approaches to enhance solubility and dissolution of fenofibrate can improve its bioavailability, both in preclinical studies and in clinical use (Guichard et al., 2000, Sauron et al., 2006, Fei et al., 2013). The ability of mesoporous silicas to enhance the solubility and absorption of poorly soluble drugs, such as fenofibrate, has been demonstrated through numerous studies in different animal models (Mellaerts et al., 2008, Van Speybroeck et al., 2010a, Kiekens et al., 2012, Bukara et al., 2016a). While these formulations have shown promise in pre-clinical studies, the development of a commercial preparation has yet to be realised and further characterisation of formulation behaviour *in vivo* is warranted (McCarthy et al., 2016).

Pigs are growing increasingly important as a preclinical species to assess biopharmaceutical aspects of drug delivery as well as forecasting absorption in humans. The comparable physiology in the GIT, particularly the comparable intestinal anatomy, physiology and transit, makes pigs a useful translational model in preclinical studies (Sjogren et al., 2014).

In a previous investigation, a combination of fenofibrate-loaded mesoporous silica with HPMCAS added in a 4:1 ratio demonstrated excellent improvement in the dissolution of fenofibrate in a simulated intestinal environment (Dressman et al., 2016). The current study expands on this previous *in vitro* work and aims to explore the use of mesoporous silica with a precipitation inhibitor in a porcine model. To our knowledge, this is the first time that the absolute bioavailability has been determined

for a mesoporous silica-based formulation delivered with a precipitation inhibitor in a large animal model. The absorption of fenofibrate from the optimised silica formulation administered in both capsule and suspension forms was compared to that of a simple fenofibrate/HPMCAS mixture. Hence, in the current study, we have attempted to expand on the existing body of knowledge regarding the combined use of mesoporous silica with precipitation inhibitors *in vivo*.

Materials and methods

Chemicals and materials

Fenofibrate was purchased as crystalline drug from D.K. Pharmachem Pvt. Ltd., Mumbai, India. Porous silica powder (Parteck® SLC 500) was kindly donated by Merck KGaA, Darmstadt, Germany. HPMCAS-HF was purchased from Shin-Etsu, Tokyo, Japan. FaSSIF Powder (formerly known as SIF Powder) was kindly donated by Biorelevant.com (London, UK). Mannitol (Parteck® M 200) was purchased from Merck KGaA, Darmstadt, Germany. HPMC Capsules (Size 00) were obtained from Capsugel, Morristown, NJ, USA. Fenofibric acid and sulindac were purchased from Sigma-Aldrich Ireland Ltd., Wicklow, Ireland. All other chemicals were of analytical grade.

Preparation of prototype formulations

Preparation of fenofibrate loaded Parteck® SLC

Fenofibrate silica formulations were prepared by the solvent impregnation method according to a previously published method (Dressman et al., 2016). Briefly, a measured amount of fenofibrate was dissolved in acetone and added dropwise to the silica powder to ensure even spreading of the drug solution on the silica while avoiding excessive wetting and aggregation (Alcalá and Real, 2006). The organic solvent was simultaneously evaporated under continuous stirring. The addition and evaporation process was repeated until the loading was complete, i.e. 29% w/w. In order to remove residual solvent from the pores, the fenofibrate loaded silica was subjected to overnight drying at 50°C.

Preparation of formulations

For further evaluation, the fenofibrate-loaded silica (FF-SLC) was blended with HPMCAS (12.5% w/w), mannitol (30% w/w) and $NaHCO_3$ (7.5% w/w) using a Turbula mixer. Samples of the resultant mixture (corresponding to 67mg of fenofibrate) were filled into size 00 HPMC capsules. Mannitol and $NaHCO_3$ were included to ensure rapid disintegration of the capsule shell and to avoid clumping during drug release. The capsule formulation will be subsequently referred to as the FF-SLC: HPMCAS (4:1) capsule. Dissolution studies were also performed with this powder blend, by emptying the capsule contents directly into the dissolution medium. This formulation will be subsequently referred to as the FF-SLC: HPMCAS (4:1) powder. A suspension formulation of the fenofibrate-loaded silica was additionally prepared, by preparing a mixture of four parts fenofibrate-loaded (29% w/w) silica with one part HPMCAS and dispersing the mixture in distilled water to attain a final volume of 25ml. This formulation is subsequently referred to as the FF-SLC: HPMCAS (4:1) suspension. Samples of the physical mixture of pure crystalline fenofibrate (80% w/w) and HPMCAS (20% w/w) corresponding to 67mg fenofibrate per sample were filled into size 00 HPMC capsules and designated as the reference formulation. The compositions of the formulations evaluated in the dissolution and *in vivo* studies are summarised in table 2-1.

Table 2-1 Composition of dosage forms

Formulation Components	Formulation composition (%)			
	FF-SLC: HPMCAS (4:1) Capsule	FF-SLC: HPMCAS (4:1) Suspension	FF-SLC: HPMCAS (4:1) Powder	Reference formulation
Fenofibrate	Loaded on silica at 29%	Loaded on silica at 29%	Loaded on silica at 29%	80% as pure drug
Parteck® SLC 500 (Mesoporous silica)	50%	80%	80%	-
HPMCAS	12.50%	20%	20%	20%
Parteck® M 200 (Mannitol)	30%	-	-	-
Sodium Bicarbonate	7.50%	-	-	-
Final dosage form	HPMC Capsule (Vcaps Plus, Capsugel)	Reconstituted in 25ml water	Powder	HPMC Capsule (Vcaps Plus, Capsugel)

In vitro dissolution using biorelevant media

Considering the solubility data for pure fenofibrate in various biorelevant media and previous characterisation studies, a medium representing upper GI conditions in the fasted state was chosen for the in vitro dissolution studies (Dressman et al., 2016, Juenemann et al., 2011). FaSSIF was prepared by dissolving the appropriate amount of FaSSIF Powder in a phosphate buffer with a pH of 6.5, as described by the manufacturer (Biorelevant.com, Croydon, UK) The dissolution studies were carried out using a USP Apparatus II (AT7 Smart by Sotax, Allschwill, Switzerland) in 500ml of biorelevant medium at 37 ± 0.5°C at 75rpm. Formulations (each corresponding to 67mg of fenofibrate) were added to the pre-warmed dissolution medium. The concentration of released drug in the dissolution medium was evaluated at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105 and 120 min. Samples were automatically removed and

filtered using a CP7-35 piston pump (Sotax, Allschwill, Switzerland) and GF/D 2.7-lm filter (Whatman, Kent, UK). Measurements were carried out using a UV–vis spectrophotometer (Specord 200 Plus by Analytic Jena, Jena, Germany) at $k = 290$nm in a continuous flow through the cuvette (2mm, QS SUPRASIL®; Hellma Analytics, Müllheim, Germany). Each test was performed in triplicate, and results were expressed as mean values together with the standard deviation (SD).

Oral bioavailability in pigs

All experimental procedures were approved and performed in accordance with licences issued by the Department of Health, Ireland (project licence B100/2877) as directed by the Cruelty to Animals Act Ireland and EU Statutory Instruments. Local ethical approval was granted by University College Cork Animal Experimentation Ethics Committee (AEEC). Oral bioavailability studies were conducted as previously described (Griffin et al., 2014, O'Shea et al., 2015). Briefly, six male Landrace pigs (12.5–16kg, mean 14.5kg) were studied. On day 1, an indwelling intravenous (i.v.) catheter was inserted into the jugular vein, under general anaesthesia. During the study, pigs were fed approximately 175g of standard weanling pig pellet feed twice daily. The final feed was given 24 hours prior to dosing. As part of the study design any remaining food was to be removed 16 hours before dosing, however no food remained at this point in any of the study legs. On day 3, following an overnight fast of 16 hours, pigs were administered either FF-SLC: HPMCAS (4:1) capsule, FF-SLC: HPMCAS (4:1) suspension or reference formulation capsule, as part of a partially randomised three-way crossover study design. To facilitate handling during oral dosing, an intramuscular dose of ketamine (5mg/kg) and xylazine (1mg/kg) was

76

administered, serving as a mild relaxant. Capsule formulations were administered

with the aid of a dosing gun, after which the pigs received ≈50mL of tap water *via*

syringe. The suspension was reconstituted by mixing the appropriate quantity of

formulation blend in 25mL of tap water in a dosing syringe, which was then

administered to the pigs. A further 25mL of tap water was used to ensure that the

whole dose was administered. A washout period of 6 days was observed between

each of the three study legs. After dosing, pigs were returned to their pens and blood

samples (4mL) were collected at time zero (pre-dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5,

4, 5, 6, 8, 10, 12 and 24 hr post dosing. Water was available *ad libitum* throughout

the study period, and the animals were fed 8hr post-dose. All blood samples were

collected in heparinised tubes (Sarstedt, Germany) and immediately centrifuged at

3220g for 5min at 4°C (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf

AG, Hamburg, Germany). Plasma was collected and stored at -80°C until analysis by

HPLC.

Quantitative analysis of fenofibric acid

The pharmacokinetic evaluation of fenofibrate was based on the quantification of

fenofibric acid, the major active metabolite of fenofibrate, using a validated HPLC-UV

method, as previously described (Griffin et al., 2014). Briefly, 0.5mL plasma was

spiked with 20μL of a sulindac 100μg/mL solution in methanol as an internal

standard. Proteins were precipitated through addition of 0.5mL of 25% NaCl solution

and 1mL of 1% H_3PO_4 in methanol with thorough mixing. Samples were centrifuged

at 11,500g for 9 min (Hermle z233 M-2 fixed angle rotor centrifuge; HERMLE

Labortechnik GmbH, Wehingen, Germany). The clear supernatants were injected

onto a Synergi Fusion C18 reversed phase column (250 x 4.6mm, 4μm) (Phenomenex Inc., Macclesfield, UK) using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of 80% methanol: 20% water (adjusted to pH 2.5) at a flow rate of 1mL/min, resulting in elution of fenofibric acid and fenofibrate at 6.5 and 10.5min, respectively. UV detection was performed at 286nm. The analysis showed linearity over the range of 50–2000ng/mL with an LOQ of 80ng/mL and extraction recoveries were ≥95%.

Pharmacokinetic data analysis

The AUC for fenofibric acid after oral administration was calculated using Prism (ver. 5; GraphPad Software Inc., La Jolla, CA, USA). The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs time profiles. The absolute bioavailability (F_a) was calculated according to equation 2-1 below:

$$Fa = \left(\frac{AUC_{oral}}{AUC_{i.v.}} \right) \left(\frac{Dose_{i.v.}}{Dose_{oral}} \right) \qquad (2\text{-}1)$$

The i.v. pharmacokinetic measurements were obtained from a study conducted under similar conditions, which has previously been reported (O'Shea et al., 2015). The intravenous pharmacokinetic parameters were fitted to a two-compartment model using the PKPlus™ module in Gastroplus™ (ver. 8.6; Simulations Plus Inc., Lancaster, CA, USA) and are summarised in table 2-2. All pharmacokinetic parameters are reported as mean ± SD, with the exception of T_{max} which is reported as median (range).

Statistical analyses

One-way ANOVA was used to determine the statistical significance of calculated *in vivo* bioavailability and C_{max}. Tukey's post hoc test was used to identify pairwise statistical significance. The Kruskal–Wallis rank test, with Dunn's multiple comparison using rank sums, was used to determine the significance of differences in T_{max}. All statistical analyses were performed using GraphPad™ Prism version 5, utilising the $P < 0.05$ significance level.

***In vitro- in vivo* relationships**

The dissolution efficiency for each formulation was calculated from *in vitro* dissolution data to facilitate comparison of *in vitro* and *in vivo* data. Dissolution efficiency was calculated according to equation 2-2 below, as derived by Khan (Khan, 1975);

$$Dissolution\ Efficiency\ (D.E.) = \frac{\int_0^t y.dt}{y_{100}.t} . 100\% \tag{2-2}$$

where *y* is the percentage dissolved drug product at time *t*, and y_{100} is the area of the rectangle described by 100% dissolution at *t*. Percent bioavailability and dissolution efficiency were correlated linearly. *In vitro* dissolution efficiency was considered the explanatory (*x*) variable, while *in vivo* bioavailability was considered the response (*y*) variable. Correlation was quantified by calculating Pearson's correlation coefficient (r) using equation 2-3 below;

$$r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2} . \sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}} \tag{2-3}$$

Results

Dissolution studies

The reference formulation, filled into a capsule shell, the FF-SLC: HPMCAS powder, the FF-SLC: HPMCAS (4:1) capsule and the FF-SLC: HPMCAS (4:1) suspension were all subjected to dissolution studies. The FF-SLC: HPMCAS (4:1) suspension was prepared by simple reconstitution immediately before evaluation, as was the case in the *in vivo* studies. The reference formulation was compared to the fenofibrate-loaded silica formulations to determine the influence of the mesoporous silica on the release characteristics. The FF-SLC: HPMCAS formulation was also studied without the capsule (by emptying the capsule directly into the dissolution medium) to determine whether the capsule shell had any influence on the release kinetics. Results of the dissolution studies are shown in figure 2-1.
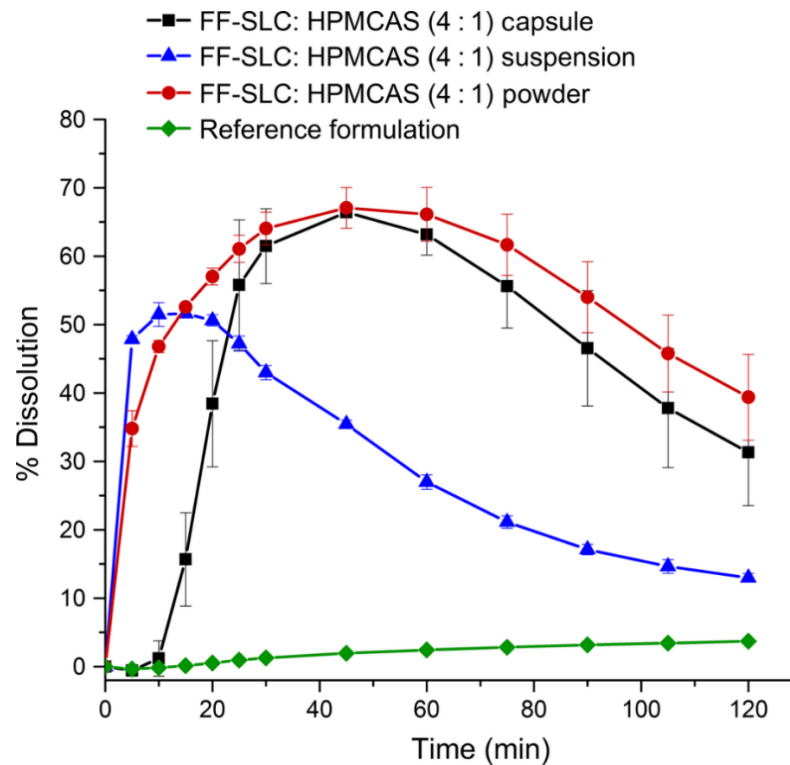
Figure 2-1 Release of fenofibrate from FF-SLC HPMCAS (4:1) capsules, FF-SLC HPMCAS (4:1) suspension, FF-SLC: HPMCAS (4:1) powder and reference formulation (each unit corresponding to 67mg fenofibrate) in fasted state simulated intestinal fluid (FaSSIF) (mean ± SD, n = 3).

Dissolution of fenofibrate from the reference formulation in FaSSIF was poor, with less than 5% of the total drug released over a period of two hours. This formulation was also unable to generate any supersaturation. These results were in good agreement with previously reported solubility data of pure fenofibrate in biorelevant media (Juenemann et al., 2011). The results reflect the poorly soluble, non-ionisable and lipophilic characteristics of fenofibrate, which consequently has very low solubility in media representing the fasted state (e.g. FaSSGF and FaSSIF) (Dressman et al., 2016). During previous investigations, dissolution of the pure drug in FaSSGF and FaSSIF-V2 had revealed that less than 1% of the dose of fenofibrate dissolved within 2hr and that even the marketed product TriCor® (which contains nanosized fenofibrate 145 mg) released less than 2% during a two-hour experiment (Dressman

et al., 2016). The slightly higher % release observed with the reference formulation compared to the earlier results can be attributed to (1) the lower dose applied and (2) the presence of the HPMCAS in the formulation. All formulations containing mesoporous silica achieved far better dissolution of fenofibrate compared to the reference formulation. Dissolution profiles for both FF-SLC: HPMCAS (4:1) powder and FF-SLC: HPMCAS (4:1) capsule reached drug concentrations corresponding to around 65% release. The slight delay for drug release from FF-SLC: HPMCAS (4:1) capsule formulation in comparison with the FF-SLC: HPMCAS (4:1) powder was attributed to the time required for disintegration of capsule shell. Further, the FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) powder formulations were effective at keeping fenofibrate in solution. The dissolution profile of the FF-SLC: HPMCAS (4:1) suspension in FaSSIF differed from those of the FF-SLC: HPMCAS (4:1) powder and the FF-SLC: HPMCAS (4:1) capsule formulation, showing slightly faster initial drug release but a lower maximum % release of fenofibrate, that is $52 \pm 0.42\%$. Moreover, the suspension formulation was less able to maintain fenofibrate at a supersaturated concentration in the dissolution medium, with the concentration declining to only 13% remaining in solution after 2 hours. Despite the excellent improvement in the dissolution characteristics achieved with the silica formulations, 100% release of fenofibrate was not achieved under fasted state conditions by any of the formulations.

Oral bioavailability in pigs

The oral bioavailability of the reference formulation, FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) suspension formulations was determined using a pig model. Figure 2-2 displays the plasma concentration–time profile following oral administration of 67mg of fenofibrate as each of these formulations to fasted pigs. Absolute bioavailability was calculated utilising i.v. pharmacokinetic data from a similar, previously reported study (Griffin et al., 2014, O'Shea et al., 2015). The key pharmacokinetic parameters are summarised in Table 2-2.
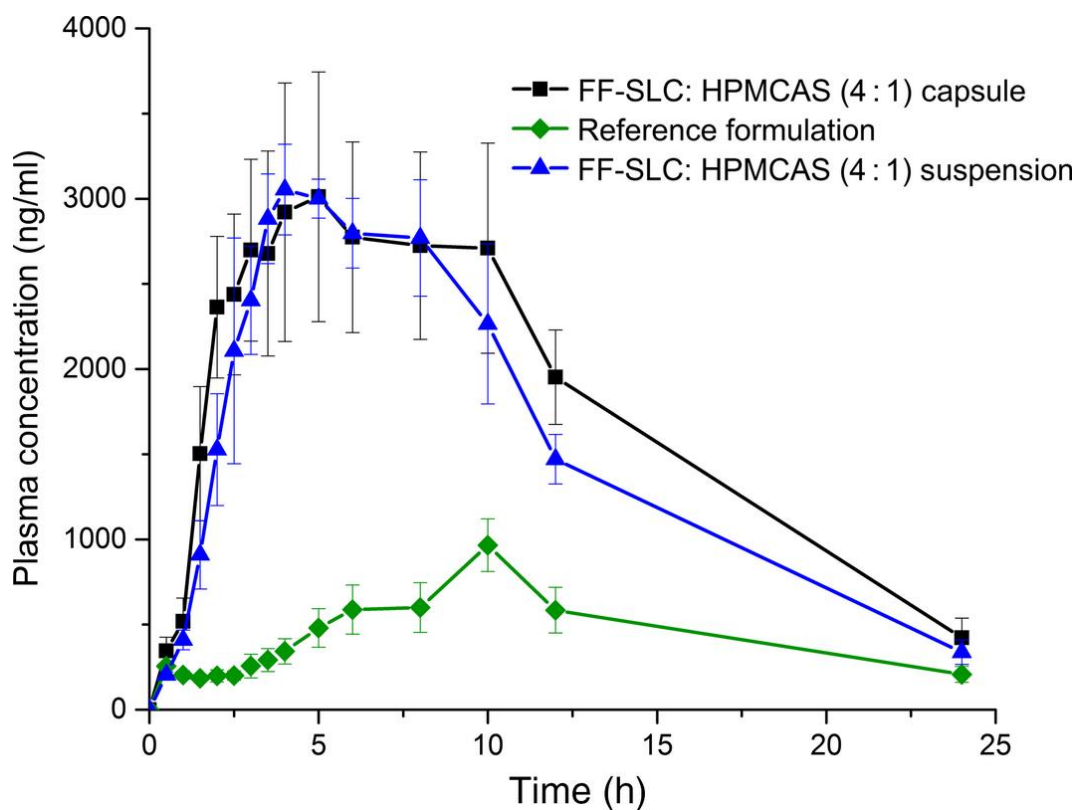
Figure 2-2 Plasma concentration of fenofibric acid vs time profiles after oral administration of 67mg fasted pigs (mean ± SE, n = 6), (■) indicates FF-SLC : HPMCAS (4:1) capsule, (◆) indicates the reference capsule and (▲) indicates silica FF-SLC : HPMCAS (4:1) suspension.

Intravenous pharmacokinetic Parameters*		Oral pharmacokinetic parameters			
V_c (L/kg)	0.345 ± 0.02504		FF-SLC: HPMCAS (4:1) capsule	FF-SLC: HPMCAS (4:1) suspension	FF: HPMCAS (4:1) reference capsule
K_{el} (hr^{-1})	0.221 ± 0.064428	C_{max} (ng/mL)	3294 ± 1614	3512 ± 863	890 ± 433
K_{el} (hr^{-1})	0.099 ± 0.038687	T_{max}** (hours)	4.5 (2.5-6)	4.5 (2.5-10)	10 (8-12)
K_{el} (hr^{-1})	0.35125 ± 0.241289	Bioavailability (%)	86.69 ± 35.37	75.47 ± 14.58	19.92 ± 9.89
$AUC_{0 \rightarrow 24hrs}$ (ng.h/ml)	18382 ± 4591	$AUC_{0 \rightarrow 24hrs}$ (ng.h/ml)	42705 ± 17422	37178 ± 7184	9815 ± 4871

Absorption from the reference formulation was slow and incomplete with an overall bioavailability of 19.92 ± 9.89% and a maximal plasma concentration of 890 ± 433ng/mL occurring at a median T_{max} of 10hr. Absorption from both mesoporous silica formulations showed a marked improvement in bioavailability compared to the reference formulation. A significant increase in overall absorption was evident (P = 0.0003) for both the FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) suspension formulations, with bioavailabilities of 86.69 ± 35.37% and 75.47 ± 14.58%, respectively. Significant increases were also demonstrated for peak plasma concentrations, as represented by C_{max} (P = 0.0013) and rate of absorption, as

demonstrated by a reduction in T_{max} (P = 0.0066), respectively. *Post hoc* analysis revealed no significant differences in rate or extent of bioavailability between both the FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) suspension formulations. A summary of these analyses is represented graphically in figure 2-3.
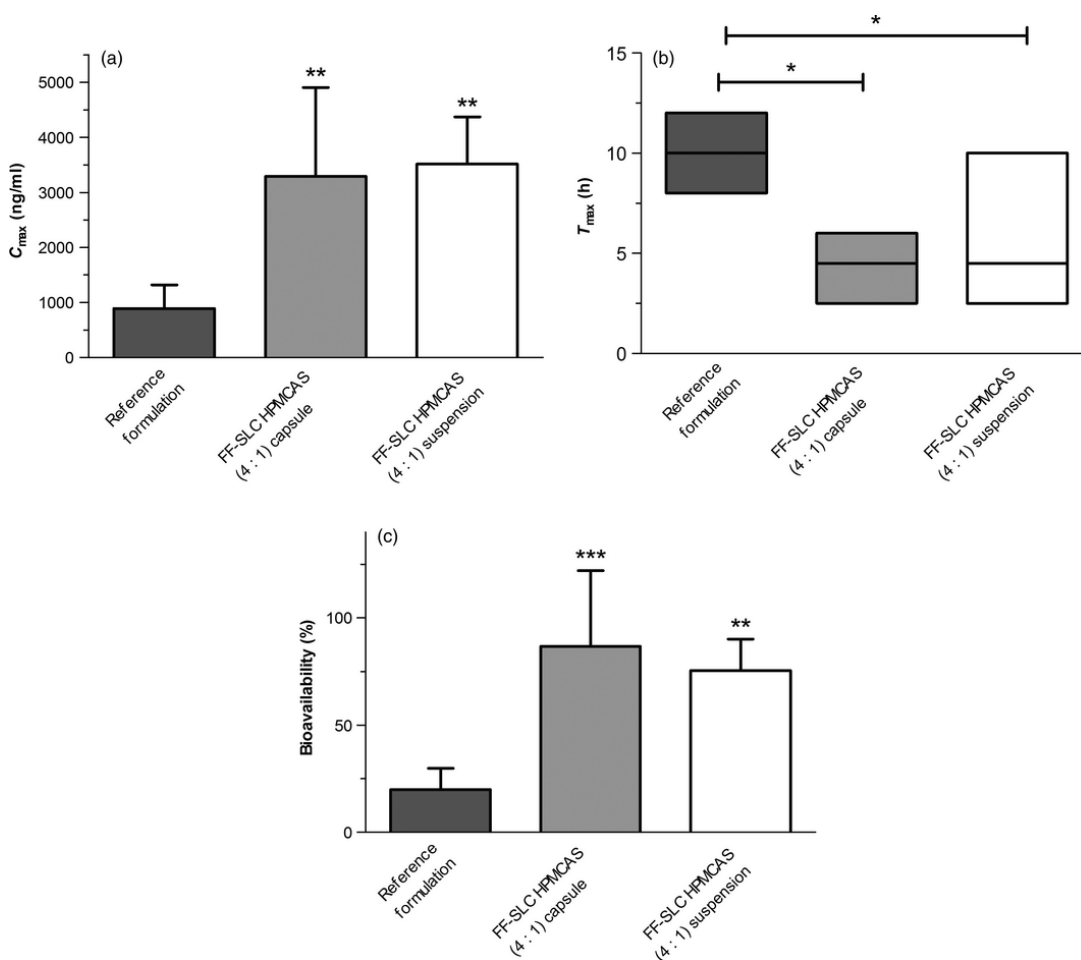

Figure 2-3 Summary pharmacokinetics of fenofibrate from FF-SLC: HPMCAS (4:1) capsules, FF-SLC: HPMCAS (4:1) suspension and the reference formulation. (a) Displays C_{max} (mean ± SD, n = 6), (b) displays T_{max} (median, range, n = 6) and (c) displays bioavailability, (mean ± SD, n = 6). Statistical significance represented in comparison with the reference formulation. Pairwise comparison of capsule and FF-SLC: HPMCAS (4:1) suspension formulations was nonsignificant in all cases.

In vitro- in vivo relationship

In the current study, single-point correlations for the fenofibrate-loaded silica formulations and the reference formulation using the mean data values obtained from pharmacokinetic and *in vitro* dissolution data were evaluated. The dissolution efficiency (DE), calculated as described in the method section, was correlated with the absolute bioavailability. Figure 2-4 shows that a high level of correlation exists between DE (%) and Fa (%) values for the silica formulations (Pearson's correlation coefficient; *r* = 0.98). This implies that increases in *in vitro* dissolution of fenofibrate from silica formulations are translated into enhancement of *in vivo* dissolution and therefore oral bioavailability. However, for quantitative predictions of absorbed fraction *in vivo*, further data generation and/or application of physiologically based pharmacokinetic modelling would be necessary.
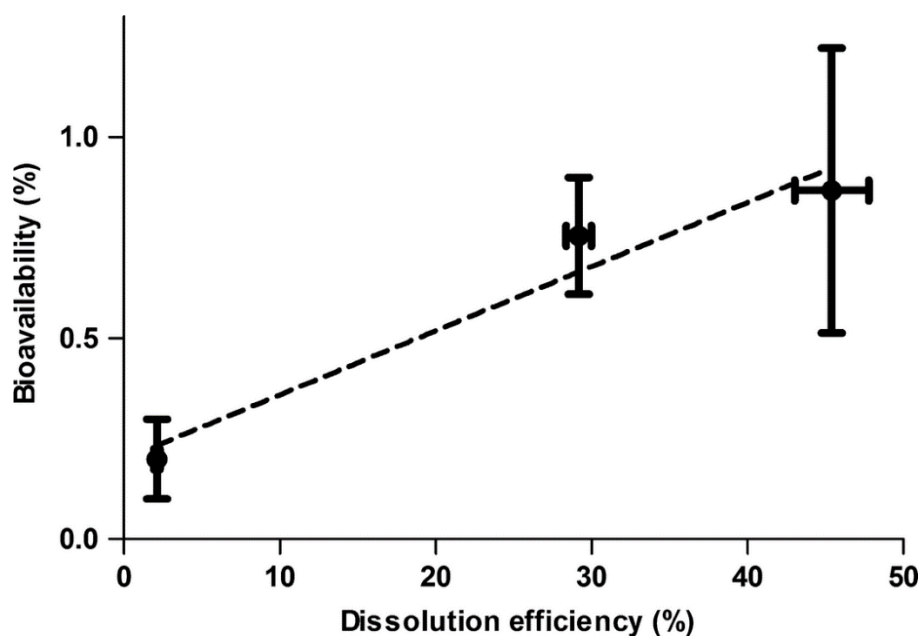

Figure 2-4 Correlation of bioavailability (mean ± SD, n = 6) and dissolution efficiency (mean ± SD, n = 3); Pearson's correlation coefficient; r = 0.98.

Discussion

Dissolution studies

In a previous investigation, the potential of mesoporous silica in combination with polymeric precipitation inhibitors to improve the *in vitro* release of fenofibrate was evaluated in biorelevant dissolution tests (Dressman et al., 2016). It was observed that this formulation strategy not only substantially improved the dissolution profile of fenofibrate under fasted state conditions but also helped to sustain supersaturated concentrations of fenofibrate, compared to either pure drug or the marketed product (TriCor®145 mg) (Dressman et al., 2016). The current study was carried out to determine whether this extraordinary enhancement in fenofibrate release observed with the optimised silica formulation could be translated into improved *in vivo* performance. The influence of dosage form on the release profile was assessed by *in vitro* dissolution before carrying out *in vivo* studies. Compared to classical quality control media, biorelevant media resemble the fasted state intestinal conditions more accurately and, hence, can provide better possibility to predict the drug release in upper GI tract (Jantratid et al., 2008). Therefore, in this study, biorelevant media resembling the fasted state in the intestine (FaSSIF) were used to obtain a better understanding of how the different dosage forms of optimised blend of mesoporous silica would release fenofibrate. Similar to the previous study, all mesoporous silica formulations showed far higher release profiles than the drug/HPMCAS mixture in FaSSIF, suggesting that there would be a substantial improvement in the bioavailability. The FF-SLC: HPMCAS (4:1) suspension showed a very rapid drug release in FaSSIF, displaying a pronounced 'spring effect'. However,

the maximum % release achieved was lower than for the powder and capsule formulations, and the maintenance of supersaturation was poorer, leading to a limited 'parachute effect'. This is most likely due to high levels of supersaturation being transiently generated in preparing the suspension formulation, where the dose is suspended in a 25ml volume of water immediately before the addition to the 500ml dissolution vessels. Such high levels of supersaturation in the suspension increase the risk of precipitation. In the case of the capsule, gradual disintegration in the larger (500ml) volume is less likely to result in such high levels of supersaturation and the risk of precipitation is lower. Dissolution profiles of both the powder and capsule versions of the FF-SLC: HPMCAS (4:1) formulation reached their highest levels after the first few sampling time points. Apart from a slightly later onset of drug release from the capsule, both formulations showed comparable dissolution profiles. It was concluded that the release of fenofibrate from the optimised blend of mesoporous silica in small intestine would not be negatively influenced by filling the powder blend into a capsule shell. Both solid formulations successfully maintained supersaturation of released fenofibrate up to 2hr during release studies, indicating strong potential to act as both a 'spring' and a 'parachute'. Generation and maintenance of supersaturation is expected to boost the absorption and hence the bioavailability of poorly soluble drugs (Brouwers et al., 2009). Therefore, based on results of dissolution tests in biorelevant media, one would predict that the capsule formulation has better potential to improve the bioavailability of fenofibrate than the suspension or reference formulations.

***In vivo* studies**

This bioavailability enhancing potential was subsequently assessed in a pharmacokinetic study in fasted pigs. Fenofibrate absorption and bioavailability has been well characterised and is typical of a BCS class II compound, with conventional formulations of fenofibrate displaying low and variable bioavailability in the fasted state due to low solubility and resultant slow dissolution (Miller and Spence, 1998). Fenofibrate bioavailability increases significantly with increased solubilisation and/or dissolution, as demonstrated by the considerable increases in absorption when delivered either in the fed state or in dissolution enhancing formulations (Sauron et al., 2006). In the current study, a blend of crystalline fenofibrate with HPMCAS displayed slow (T_{max} 8–12 hr) and incomplete absorption (Fa = 19.92 ± 9.89%) which is in line with both previous and current *in vitro* dissolution studies. The poor absorption of this reference formulation was predicted well by the poor dissolution performance demonstrated *in vitro* in both this and previous studies (DE = 2.13 ± 0.19%) (Dressman et al., 2016). Both the rate and extent of bioavailability were significantly increased by delivery of the fenofibrate-loaded silica formulated with HPMCAS. The supersaturation observed *in vitro* appears to have been replicated *in vivo* with almost complete absorption from both the FF-SLC: HPMCAS (4:1) capsule (Fa = 86.69 ± 35.37%) and FF-SLC: HPMCAS (4:1) suspension formulation (Fa =75.47 ± 14.58%). These results compare favourably to other formulations previously assessed in fasted pigs including a commercial nanosized preparation (Lipantil Supra®; 71.08 ± 25.78%), a commercial micronised capsule (Lipantil® Micro; 66.1 ± 3.5%) and novel lipid based formulation (lipidic dispersion; 60.3 ± 8.2%) (McCarthy et al., 2017, O'Shea et al., 2015). Rapid dissolution also appears to have occurred,

89

with T_{max} reduced to a median of 4.5 hr for both the FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) suspension formulations, respectively. This improved *in vivo* dissolution was anticipated based on the enhanced dissolution performance demonstrated *in vitro* in dissolution studies of the FF-SLC: HPMCAS (4:1) capsule (DE = 45.41 ± 2.37%) and FF-SLC: HPMCAS (4:1) suspension formulations (DE = 29.18 ± 0.80%) in FaSSIF. As the reference formulation and the two silica-based formulations both contained HPMCAS in the same quantity, it can be additionally concluded that the drug dissolution and enhanced drug absorption can be mainly attributed to use of mesoporous silica.

Conclusions

The combination of fenofibrate-loaded silica with HPMCAS as a polymeric precipitation inhibitor (FF-SLC: HPMCAS (4:1) capsule and FF-SLC: HPMCAS (4:1) suspension formulations) achieved substantial improvements in bioavailability in fasted pigs compared to a mixture of crystalline fenofibrate with HPMCAS. Biorelevant *in vitro* dissolution data provided a useful indicator of formulation performance *in vivo*, with enhanced dissolution and supersaturation *in vitro* transferring into improvements in bioavailability in the *in vivo* studies. The results confirm the ability of this formulation strategy to improve biopharmaceutical performance of fenofibrate in higher animal models, and further raises the prospects of a commercial silica-based formulation in the near future.

Chapter 3 : Lipidic dispersion to reduce food dependent oral bioavailability of fenofibrate: *in vitro*, *in vivo* and *in silico* assessments

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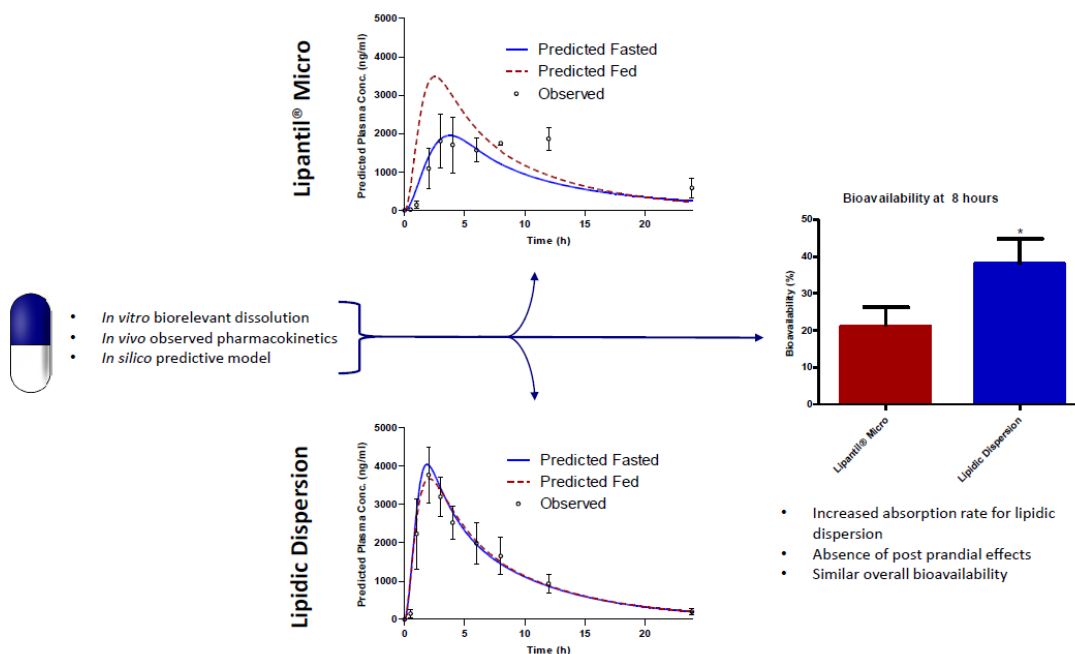
Graphical abstract

Figure 3-1 Graphical abstract

Abstract

Novel formulations that overcome the solubility limitations of poorly water soluble drugs (PWSD) are becoming ever more critical to a drug development process inundated with these compounds. There is a clear need for developing bioenabling formulation approaches to improve oral bioavailability for PWSD, but also to establish a range of predictive *in vitro* and *in silico* biopharmaceutics based tools for guiding formulation design and forecasting *in vivo* effects. The dual aim of this study was to examine the potential for a novel lipid based formulation, termed a lipidic dispersion, to enhance fasted state oral bioavailability of fenofibrate, while also assessing the predictive ability of biorelevant *in vitro* and *in silico* testing. Formulation as a lipidic dispersion improved both dissolution and solubilisation of fenofibrate through a combination of altered solid state characteristics and incorporation of

solubilising lipidic excipients. These changes resulted in an increased rate of absorption and increased maximal plasma concentrations compared to a commercial, micronised product (Lipantil® Micro) in a pig model. Combination of biorelevant *in vitro* measurements with *in silico* physiologically based pharmacokinetic (PBPK) modelling resulted in an accurate prediction of formulation performance and forecasts a reduction in food effects on fenofibrate bioavailability through maximising its fasted state dissolution.

Introduction

Designing novel formulations to enhance the oral bioavailability of poorly water soluble drugs has long been a key driver of the pharmaceutical industry. The poor intrinsic solubility of Biopharmaceutical Classification Scheme (BCS) class II compounds has stifled development of many emerging therapeutic compounds. With up to 75% of drug development candidates displaying poor aqueous solubility, the bioavailability limitations posed still form an unmet challenge for pharmaceutical drug development (Di et al., 2012).

The absorption of these poorly water soluble drugs (PWSD) is limited by their poor solubility and resultant slow dissolution rate within gastrointestinal fluid (Butler and Dressman, 2010). In addition, these drugs can commonly display variable food effect bioavailability, with poor solubility being a strong predictor of positive food effects (Bergstrom et al., 2014, Lentz, 2008). Ingested lipids interact with bile salts and phospholipids in the post-prandial intestinal milieu to solubilise PWSD (Charman et al., 1997). While this can enhance absorption of PWSD, it can also lead to variable bioavailability during clinical use depending on the prandial state at the time of dose administration, potentially resulting in loss of efficacy (Custodio et al., 2008). Formulations that enhance bioavailability of these compounds, maximising it in the fasted state, will therefore result in reduced food effects (Sauron et al., 2006, Guichard et al., 2000).

Formulation techniques that enhance bioavailability of PWSD in a predictable and reproducible manner are becoming increasingly critical. The design of these bioenabling formulation approaches can be described using the concept of the

"spring and parachute" approach (Guzman et al., 2007). Facilitation of dissolution is thought of as providing an initial "spring", while inclusion of solubilising excipients or precipitation inhibitors can act as a "parachute", retarding the transition back to a lower energy, crystalline form. Critically, the selection of formulation methods and/or excipients to maximise oral bioavailability is best guided by reliable and predictable *in vitro* biopharmaceutical screening.

The advent of Developability Classification System (DCS), based on a revised BCS, has placed greater focus on understanding of the factors affecting drug and formulation performance *in vivo*. By sub-dividing BCS class II compounds into class IIa and IIb, based on more biorelevant screening, the DCS enables earlier prediction of drug limitations in development, guides formulation strategy and can be used to estimate formulation performance (Butler and Dressman, 2010). Complete oral absorption for dissolution rate limited (class IIa) drugs can generally be achieved by simply controlling particle size, surface area and wettability, while solubility limited candidates (class IIb) require more complex solubilisation techniques, such as nanonisation, solid dispersion, salt or co-crystal formation or inclusion of solubilising excipients, such as lipids and surfactants (Butler and Dressman, 2010, Williams et al., 2013b).

It is also imperative when designing bioenabling formulation strategies to establish reliable *in vitro–in vivo* correlations. While solubility, dissolution and permeability tests are often of merit for conventional formulations, more advanced biorelevant screening tools and computational modelling approaches are needed for reliably predicting *in vivo* performance (Bergstrom et al., 2014). *In silico* physiologically based

pharmacokinetic (PBPK) modelling builds on the available *in vitro* data and is being increasingly used to forecast formulation and food effects. Several programs are now commercially available for model generation and application to assess *in vivo* performance, including Gastroplus™, Simcyp® and PK-Sim® (Kostewicz et al., 2014a). Combination of *in vitro* solubility, dissolution and precipitation testing with *in silico* data modelling has been shown to be particularly effective at predicting *in vivo* performance of oral dosage forms (Fei et al., 2013, Juenemann et al., 2011, Stillhart et al., 2014b).

Fenofibrate is an orally active, lipid regulating, BCS class II compound, and is a good model for the assessment of formulation strategies to enhance bioavailability and eliminate food effect (Fei et al., 2013, Juenemann et al., 2011). The Lipantil Micro® formulation, a micronised product, displays food dependent bioavailability, and therefore requires administration with food. A re-formulated product, Lipantil® Supra was developed using NanoCrystal® technology, to overcome this limitation and allows food independent administration and dose reduction (Sauron et al., 2006, Guichard et al., 2000, Junghanns and Muller, 2008).

The aim of this study was to explore an alternative bioenabling formulation approach to overcome food dependent bioavailability using lipid based formulations. Lipid based formulations (LBFs) have been widely investigated for their ability in enhancing solubilisation within the GI tract, generating supersaturation and increasing drug absorption and have been shown to eliminate food effect *in vivo* (O'Driscoll and Griffin, 2008, Christiansen et al., 2014, Williams et al., 2013c). Solubilisation of PWSD within a lipid-based, liquid carrier allows delivery within a capsule which self

emulsifies on dispersion in GI fluids, maintaining drug solubilisation. Co-administration of lipids as formulation excipients may promote formation of mixed micelles enhancing solubilisation and induce secretion of bile salts and phospholipids *in vivo*, mimicking the fed state environment (O'Reilly et al., 1994, Kossena et al., 2007).

This study has the dual objective of investigating the potential for a novel LBF, termed a lipidic dispersion, to enhance bioavailability of fenofibrate in fasted pigs, while assessing the ability of *in vitro* and *in silico* biopharmaceutical tools to predict *in vivo* formulation performance. The novel formulation is based on a modification of previous work and combines solid dispersion and lipid formulation techniques, addressing challenges associated with the delivery of dissolution rate and solubility limited drugs (Faisal et al., 2013).

Materials and methods

Chemicals and materials

Olive Oil 'highly refined, low acidity' (C_{18} triglycerides), fenofibric acid, Tween 85 (polyoxyethylene-(20)–polysorbitan trioleate), sodium taurocholate (>95%) and sodium oleate ($\geqslant$82% fatty acids, as oleic acid) were purchased from Sigma–Aldrich (Ireland). Cremophor RH 40 (polyoxyl-40-hydrogenated castor oil) and Kollidon® 30 (polyvinylpyrrolidine (PVP) K30) were received from BASF (Germany). Lipantil® Micro 67mg hard capsules were obtained from Abbott Healthcare Products Ltd. (UK). Glycerol monooleate (GMO, Rylo MG19 Pharma®, 99.5% monoglyceride) was received from Danisco Specialities (Denmark). Fenofibrate was purchased from Kemprotec Ltd. (UK). Hard gelatin capsules (Size 0) were obtained from Capsugel (Coni-Snap®). Lecithin (Lipoid E PC S, >98% pure) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). All other chemicals and solvents were of analytical grade or HPLC grade respectively and were purchased from Sigma–Aldrich (Ireland).

Preparation of fenofibrate loaded solid dispersion

A solid dispersion of fenofibrate and PVP K30, in a 1:4 ratio, was prepared using a Büchi mini spray dryer B-290 (BÜCHI labortechnik AG, Switzerland). Fenofibrate and PVP-K30 were dissolved in dichloromethane (40mg PVP/ml) and dried in an inert nitrogen atmosphere. The operating parameters were as follows: inlet temperature: 55°C, outlet temperature: 40°C, pump rate: 14% and aspiration rate: 100%. The solid dispersion was collected from the cyclone separator and stored in a desiccated environment at room temperature. Physical mixtures of the same ratios were also

prepared by mixing fenofibrate and PVP-K30 thoroughly in a mortar until a homogenous mixture was obtained.

Preparation of lipidic dispersion

An LBF composed of 40% long chain triglyceride (LCT) (Olive oil), 20% surfactant (Cremophor RH 40) and 40% co-surfactant (Tween 85) was prepared as previously described (Faisal et al., 2013). This self-emulsifying drug delivery system (SEDDS) was chosen on the basis of its high composition of LCT and the ability to form a stable, isotropically clear microemulsion on dilution with water. The SEDDS was prepared by weighing exact quantities of each excipient into a screw cap glass tube followed by vortexing to allow complete mixing and incubated overnight at 37 °C (Faisal et al., 2013). Fenofibrate and PVP (1:4) were dissolved in dichloromethane (40mg PVP/mL). Subsequently, the LBF was added to the solution and mixed using a magnetic stirrer. The total weight ratio of constituents (fenofibrate: PVP: LBF) was 1:4:5. The solution was spray dried using parameters defined in the previous section. A blank formulation was prepared under similar conditions, but without the addition of drug, with a 4:5 ratio of PVP to LBF. Both drug loaded and blank lipidic dispersions resulted in the formation of a free-flowing white powder. These formulations were stored in a desiccated environment and fenofibrate content was assayed and found to be stable over a storage period of six months. Stability was indicated by similarity in fenofibrate content assay, as determined by HPLC, at initial and six-month time-points. At the initial time-point 89.04% ± 5.56% of theoretical drug concentration was observed, compared to 91.94% ± 3.98% at the six-month time-point.

Physiochemical characterisation

Thermal analysis

Differential scanning calorimetry (DSC) analyses were carried out using a DSC Q1000 (TA Instruments, Hertfordshire, UK). Sealed samples and reference pans were loaded into the sample chamber at ambient temperature, equilibrated to 25°C and held at this temperature for 5 min. Samples were heated at 3°C/min with an applied modulation of ±1°C every 60 sec from −40 to 200°C. The nitrogen gas flow rate was 50ml/min. Analysis of the DSC thermograms was conducted with Universal Analysis 2000 software (TA Instruments, Hertfordshire, UK).

Powder X-ray diffraction

Powder X-ray diffraction (PXRD) was carried out using a Stadi MP Diffractometer (Stoe GmbH, Germany). Samples were radiated using a copper anode (Cu Kα radiation, λ = 1.5406Å, 40kV, 40mA). The scanning angle ranged from 3.55° to 60° of 2ϑ, with a scanning speed of 0.07°/sec. The diffraction patterns were analysed using Philips X'Pert High Score software (version 1.0a).

Compendial dissolution

Compendial dissolution studies were carried out in triplicate with an Erweka DT600 dissolution test system (Erweka GmbH, Germany). Tests were performed in 900 ml 0.05M sodium lauryl sulphate (SLS) at 37 ± 0.5°C using USP type II paddle method at 75rpm. Samples equivalent to 67mg of fenofibrate were placed in the dissolution medium within gelatin capsules, using wire sinkers. Samples of 4ml were withdrawn at 5, 15, 30, 45, 60 and 90 min, immediately followed by the addition of an equal volume of fresh, pre-warmed medium. The withdrawn samples were filtered through

100

a 0.20μm PES membrane filter (Filtropur S 0.2, Sarstedt AG & Co., Nümbrecht, Germany), discarding the first 2ml. The resultant filtrate was visually assessed as being clear and free from particles. 100μL of sample was immediately diluted with 900μL of acetonitrile and analysed using HPLC.

Biorelevant solubility and dissolution

FaSSIF-V2 and FeSSIF-V2 were prepared as outlined in the literature (Jantratid et al., 2008). Enhanced FaSSIF-V2 and FeSSIF-V2, containing formulation excipients, were prepared by running a simulation of dissolution conditions outlined below with blank spray dried lipidic dispersion in biorelevant medium, in a quantity equivalent to that contained in biorelevant dissolution test samples.

Solubility studies were carried out by the addition of excess fenofibrate to biorelevant media and using a standardised shake flask method with a shake time 24 hr at 37°C (Juenemann et al., 2011). 2 ml samples were removed at 24 hr and added to 2ml centrifuge tubes. Samples were centrifuged at 16,500g for 13 min (Hermle z233M-2 fixed angle rotor centrifuge, HERMLE Labortechnik GmbH, Wehingen, Germany). The resultant supernatant was free from particles and was removed and centrifuged again under the same conditions. The resulting supernatant was analysed using HPLC after appropriate dilution with acetonitrile.

Biorelevant dissolution was carried out under similar conditions to those described for compendial dissolution studies with the following modifications: 500ml of biorelevant media was used, with samples withdrawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min.

Oral bioavailability in pigs

The study was carried out under licences issued by the Department of Health, Ireland, as directed by the Cruelty to Animals Act, Ireland and EU Statutory Instruments. Local University ethical committee approval was also obtained. The study was a partially randomised three-way crossover design, where the pigs were randomly allocated to one of the two oral formulations on the first leg, followed by a crossover for the second leg. The final leg of the study involved an intravenous study on all pigs. The data from this intravenous study have been previously used for the determination of fenofibrate clearance in pigs to allow absolute bioavailability to be determined in a separate study (Griffin et al., 2014).

Four male landrace pigs (15–20kg, mean 17.5kg) were sourced locally and housed at the University's Biological Services Unit. Pigs were fasted for 16 hr before experiments. On day 1, an indwelling intravenous catheter was inserted into the jugular vein, under general anaesthesia as previously described (Faisal et al., 2013). Following an overnight fast on day 3, oral formulations were administered in gelatin capsules (equivalent to 67mg fenofibrate) with the aid of a dosing gun, after which the pigs received 50mL of water via syringe. After dosing, pigs were returned to their pens. Blood samples (4mL) were collected at time zero (pre-dosing) and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hr post-dosing. Water was available *ad libitum* throughout the study period and the animals were fed 8 hr post-dose.

For the intravenous treatment, animals were administered 25mg fenofibrate by slow infusion, over 2 min, via 3ml of a solution containing 8.33mg/ml fenofibrate in 80% w/w ethanol and 20% physiological saline into an ear vein. Blood sampling was

102

performed as outlined above, with an additional 3 blood samples taken at 0.0833, 0.25 and 0.75 hr post-dose. All blood samples were collected in heparinised tubes (Sarstedt, Germany) and immediately centrifuged at 3,220g for 5 min at 4°C (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany). Plasma was collected and stored at −80°C prior to analysis. A seven day washout period was observed between each leg of the study. All animals remained in good health throughout the study.

Quantitative analysis of fenofibrate

The concentrations of fenofibrate from solubility/dissolution tests were determined using a validated HPLC-UV method. The HPLC system comprised of an Agilent Technologies 1200 series HPLC system (Agilent Technologies, Santa Clara, Ca.) equipped with a Kinetex 5 μm XB-C18, 250 × 4.6 mm reversed phase column (Phenomenex Inc., Macclesfield, UK). A mobile phase consisting of 80% acetonitrile and 20% water was used at a flow rate of 1ml/min. An injection volume of 20μl was used. Fenofibrate was detected using UV light at 286nm, with retention time of 8 min. This analysis displayed linearity ($r^2 \geqslant 0.99$) over the range 25–5000ng/ml. The precision of the method at 25, 400 and 1500 ng/ml, expressed as the coefficient of variation (CoV), was 0.48%, 2.45% and 1.89% within days and 0.57%, 3.58% and 2.23% between days respectively.

The pharmacokinetic evaluation of fenofibrate was based on the quantification of fenofibric acid, the major active metabolite of fenofibrate, using a validated HPLC-UV method, as previously described (Griffin et al., 2014). Briefly, 0.5ml of plasma was spiked with sulindac as an internal standard. Proteins were precipitated through the

103

addition of 0.5ml of 25% NaCl solution and 1 ml of 1% H_3PO_4 in methanol with thorough mixing. Samples were centrifuged at 11,500g for 9 min (Hermle z233M-2 fixed angle rotor centrifuge, HERMLE Labortechnik GmbH, Wehingen, Germany). The clear supernatants were injected onto a Synergi, C18 reversed phase column (250 × 4.6mm, 4μm) (Phenomenex Inc., Macclesfield, UK) using the Agilent system previously described in this section. Mobile phase consisted of 80% methanol: 20% water (adjusted to pH 2.5) at a flow rate of 1 ml/min, resulting in elution of fenofibric acid and fenofibrate at 6.5 and 10.5 min respectively. UV detection occurred at 286nm. The analysis showed linearity over the range of 50–2000ng/ml with an LOQ of 80ng/ml and extraction recoveries ⩾95%.

Pharmacokinetic data analysis

Intravenous pharmacokinetic parameters were fitted to a two compartment model using the PKPlus™ module in Gastroplus™ (ver. 8.6, Simulations Plus Inc., Lancaster, Ca.). The AUC for fenofibric acid after oral administration was calculated for 8 hr and 24 hr post-dosing using Prism (ver. 5, GraphPad Software Inc., La Jolla, Ca.). The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs. time profiles. The Absolute Bioavailability (F_a) was calculated according to equation 3-1 below:

$$Fa = \left(\frac{AUC_{oral}}{AUC_{i.v.}} \right) \cdot \left(\frac{Dose_{i.v.}}{Dose_{oral}} \right) \tag{3-1}$$

All pharmacokinetic parameters are reported as mean ± SD.

In silico predictive modelling

In silico absorption modelling was conducted using GastroPlus™ (ver. 8.6, Simulations Plus, Lancaster, Ca.). The ADMET Predictor™ module was used to estimate fenofibrate physiochemical characteristics. Reference and biorelevant solubilities were changed to reflect those measured *in vitro* in aqueous, as well as biorelevant fed and fasted media. The z-factor for each formulation was established by fitting individual dissolution profiles in FaSSIF-V2 and FeSSIF-V2. Pharmacokinetic parameters and bioavailability were fit to a two-compartment model using the built-in PKPlus™ module comparing mean i.v. profile to the quickest dissolving (lipidic dispersion) formulation and subsequently optimised using the software's optimisation functionality to best fit i.v. and lipidic dispersion profiles. Simulations were based on the assumption that the entire absorbed dose was rapidly converted to fenofibric acid by gut and plasma esterases (Fei et al., 2013, Griffin et al., 2014). Simulations were set to 24 hr using the mini-pig physiological fasted ACAT™ model and incorporated a feeding step at 8 hr. Dose was set to 67mg and dose volume was 50ml. All other values were kept at default values. Simulated profiles were compared to the mean plasma profile for each formulation.

Statistical analyses

Dissolution curves obtained with each formulation were compared using the similarity factor, f_2, described by Moore and Flanner, as defined by equation 3-2 below (Moore and Flanner, 1996);

$$f_2 = 50 log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} x \; 100 \right\} \tag{3-2}$$

where n is the number of sample time-points, Rt is the percentage of drug solubilised at time t in the reference vessel and Tt is the percentage of solubilised drug at time t in the test vessel. Curves were defined as similar when $50 \leqslant f2 \leqslant 100$.

In vitro solubility data were tested for significance ($p < 0.05$) using a two-tailed, independent sample t-test, assuming Gaussian distribution and equal variance.

Paired t-tests were used to determine the statistical significance ($p < 0.05$) of calculated *in vivo* bioavailability and pharmacokinetic results, as each animal acted as its own control in this crossover study.

Correlation between predicted and observed profiles was assessed using Pearson's correlation test. Correlation between values was first assessed for linearity using a scatter plot, before calculating Pearson's *r*. Gaussian distribution was assumed. Correlation was considered significant when $p < 0.05$.

All statistical analyses were performed using GraphPad Prism version 5.

Results

Lipid excipient effects on fenofibrate solubility in biorelevant conditions

The measured solubility of fenofibrate in biorelevant media (FaSSIF-V2 and FeSSIF-V2) was used as a predictor of *in vivo* solubility at the absorptive site. The effect of lipidic formulation excipients on solubilisation was assessed by their addition to these media (Enhanced FaSSIF-V2 and Enhanced FeSSIF-V2). Solubility in fasted state media was increased from 3.641 ± 0.623 µg/ml in FaSSIF-V2 to 58.173 ± 2.542 µg/ml in Enhanced FaSSIF-V2 (mean difference 41.6 ± 0.448 µg/ml, $p < 0.0001$). This was compared to an increase from 45.24 ± 0.462 µg/ml in FeSSIF-V2 to 88.829 ± 1.374 µg/ml in Enhanced FeSSIF-V2 (mean difference 30.66 ± 1.668 µg/ml, $p < 0.0001$). As a result, the ratio of fed: fasted solubility was reduced from 16 without lipids to 2 when lipids are present, demonstrating the ability of lipidic excipients to improve biorelevant solubility, while also attenuating a food effect for fenofibrate (fig. 3-2).
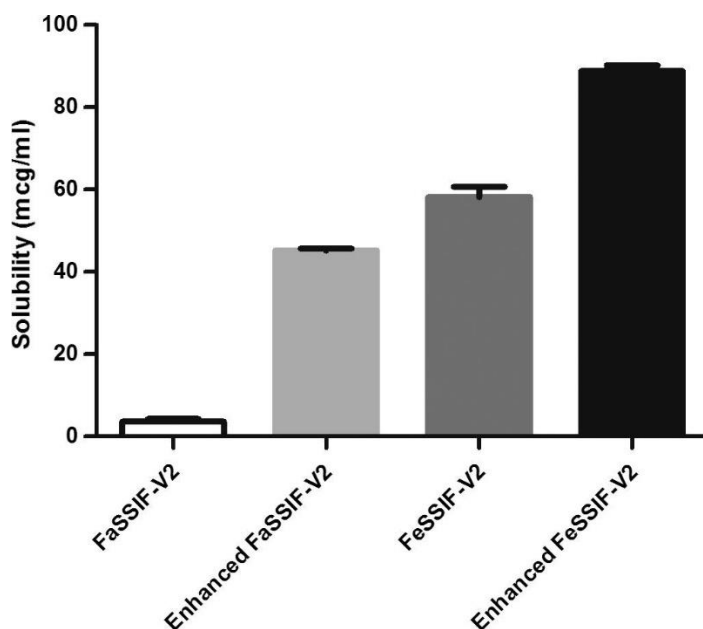

Figure 3-2 Solubility of fenofibrate in fasted state simulated intestinal fluid-V2 (FaSSIF-V2), fed state simulated intestinal fluid-V2 (FeSSIF-V2) and similar media incorporating lipidic formulation excipients (Enhanced FaSSIF-V2 and Enhanced FeSSIF-V2) (n = 3, mean ± SD)

Compendial dissolution

A range of formulations was initially screened using FDA recommended dissolution conditions, with a view to forecasting *in vivo* performance. A lipidic dispersion, prepared as outlined in methods, was compared to the commercial Lipantil® Micro formulation and a PVP solid dispersion, devoid of lipid excipients. These formulations were compared to pure, unprocessed drug substance and physical mixtures of excipients. The pure drug substance demonstrated slow, incomplete dissolution and release from fenofibrate–PVP physical mixture was similarly slow to that of unprocessed drug. The release from the PVP solid dispersion was greatly enhanced relative to the physical PVP–drug mixture, confirming improved dissolution of fenofibrate following processing via PVP solid dispersion (Hugo et al., 2013). In the case of the lipidic dispersion, a comparable and near complete release profile was observed relative to the commercial micronised product. The dissolution profiles for the PVP solid dispersion, lipidic dispersion and Lipantil® Micro were similar, with >70% dissolution observed at 45 min, which would indicate that using a compendial dissolution set-up, there was no discernible difference detected due to lipid excipients on the dissolution profile (figure 3-3).
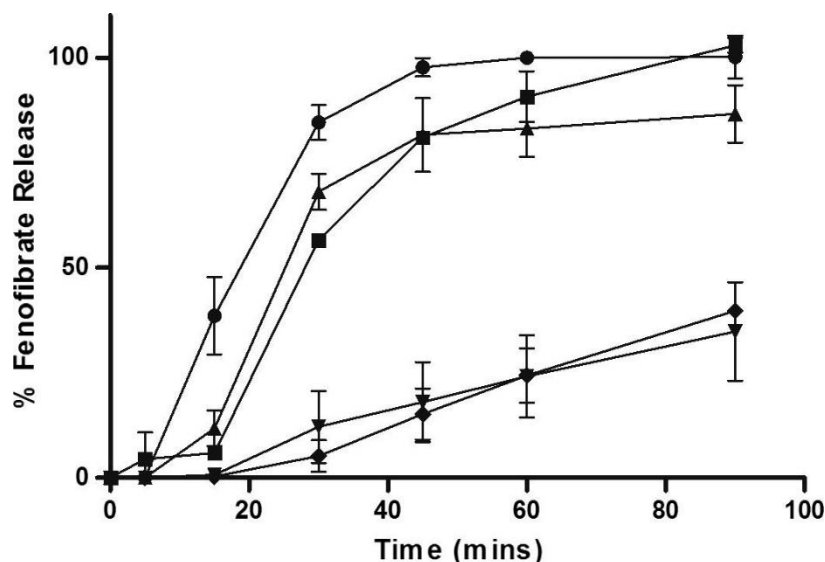
Figure 3-3 Dissolution profiles of 67 mg fenofibrate in 0.05 M SLS (n = 4, mean ± SD), (●) indicates Lipantil® Micro, (■) indicates Lipidic Dispersion, (▲) indicates solid dispersion, (▼) indicates pure fenofibrate drug substance, (◆) indicates fenofibrate and PVP-K30 physical mixture.

Physicochemical characterisation

Solid-state characterisation of the formulations was subsequently evaluated using PXRD (figure 3-4) and DSC (figure 3-5). The PXRD diffractograms of pure fenofibrate and the physical mixture of fenofibrate and PVP were in accordance with Heinz et al. (Heinz et al., 2009), with characteristic peaks of crystalline fenofibrate observed at 12° (2ϑ), 14.5° (2ϑ), 16.2° (2ϑ), 16.8° (2ϑ) and 22.4° (2ϑ). DSC investigation demonstrated a sharp melting endotherm at 77.63°C, verifying the crystallinity of fenofibrate. PXRD of Lipantil® Micro displays the characteristic peaks of crystalline fenofibrate, along with the additional peaks most likely corresponding to crystalline excipients present, such as lactose monohydrate, sodium lauryl sulphate and magnesium stearate. Although changes in heat flow are apparent, endotherms corresponding to the melting of crystalline fenofibrate are also evident for the physical mixture of excipients and Lipantil® Micro.
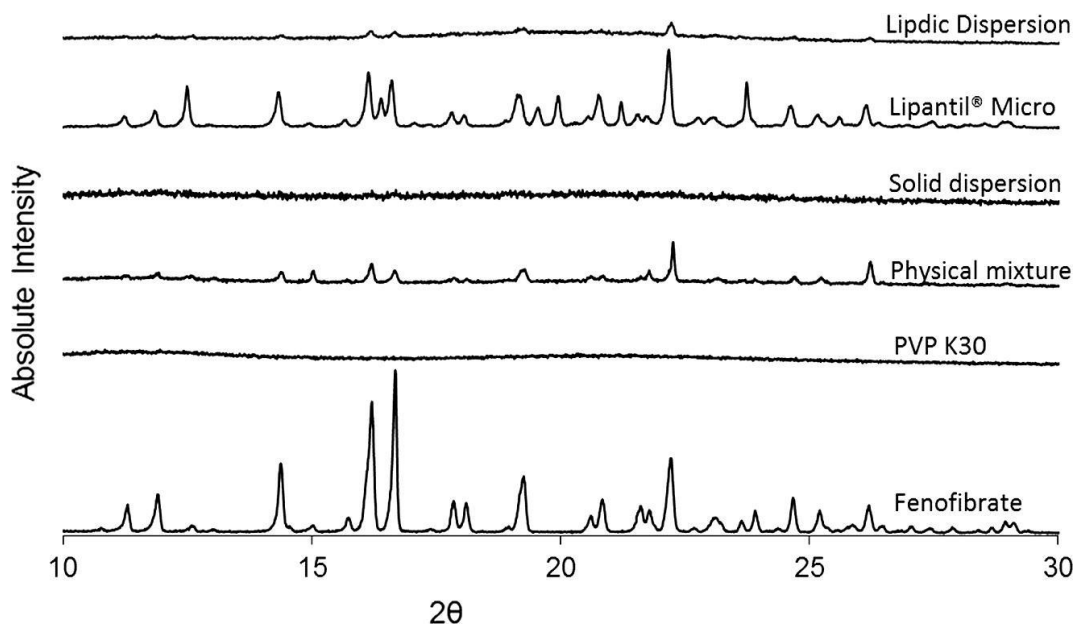
Figure 3-4 Powder X-ray diffraction patterns of fenofibrate, Lipantil® micro, PVP, fenofibrate–PVP physical mixture, fenofibrate–PVP solid dispersion and lipidic dispersion.
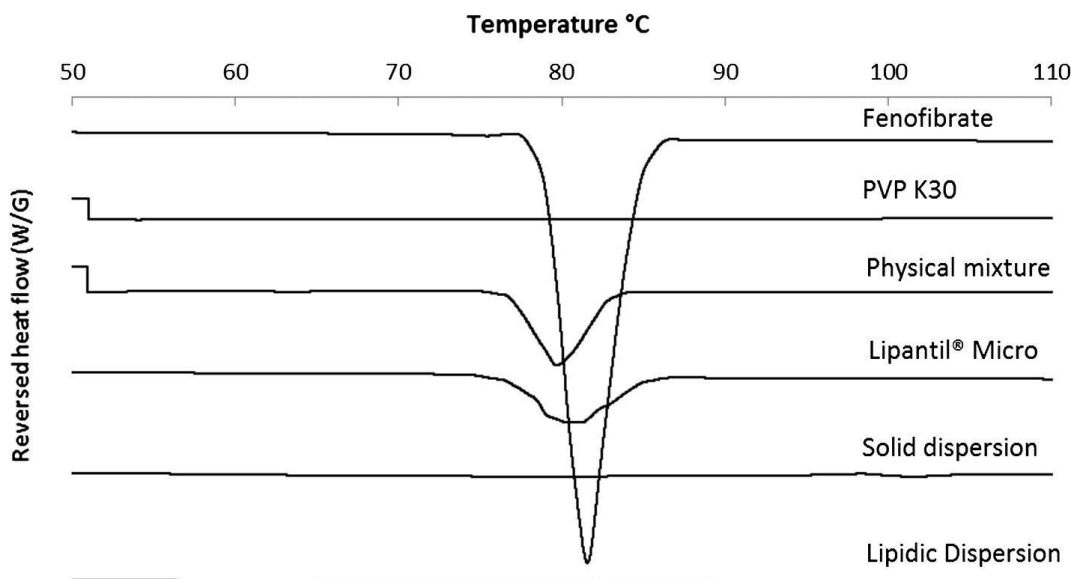

Figure 3-5 Differential scanning calorimetry thermogram of fenofibrate, Lipantil® micro, PVP, fenofibrate–PVP physical mixture, fenofibrate–PVP solid dispersion and lipidic dispersion

110

Generation of a conventional solid dispersion with fenofibrate and PVP results in a loss of crystallinity, with characteristic peaks absent and elimination of the melting endotherm. The lipidic dispersion displays similar thermal behaviour to the solid dispersion, suggesting a loss in crystallinity relative to pure drug substance. However, characteristic peaks are apparent in PXRD and there appears to be some element of crystalline fenofibrate present. Direct quantitative comparison with the physical mixture of excipients is difficult due to differences in fenofibrate content in both samples. While the fenofibrate seems to retain some crystalline character, the similar thermal behaviour to that of the solid dispersion indicates that there is no additional energy input required to break up fenofibrate crystalline lattice in the lipidic dispersion, suggesting a solid-state change which should enhance dissolution performance in a comparable fashion to solid dispersion.

Biorelevant dissolution under simulated fasting and fed state conditions

To explore the likely impact of food on dissolution characteristics, dissolution studies were conducted using biorelevant fasted and fed state media (Juenemann et al., 2011). Biorelevant dissolution testing of Lipantil® Micro demonstrates a marked increase in dissolution in the FeSSIF-V2 compared to the FaSSIF-V2 (figure 3-6A). In contrast to the compendial dissolution profile, drug release under simulated fasting conditions was low, with approximately 8% release after 2 hr. This increased substantially using FeSSIF-V2 with 52% release after 2 hr (f_2 = 27.163). In the case of the lipidic dispersion, fenofibrate release under fasting condition was significantly higher than the micronised formulation, with 43% release at 2 hr. Interestingly, there

is no difference in dissolution of the lipidic dispersion in fasted or fed state media (figure 3-6B) with a similarity factor (f_2) of 58.198.
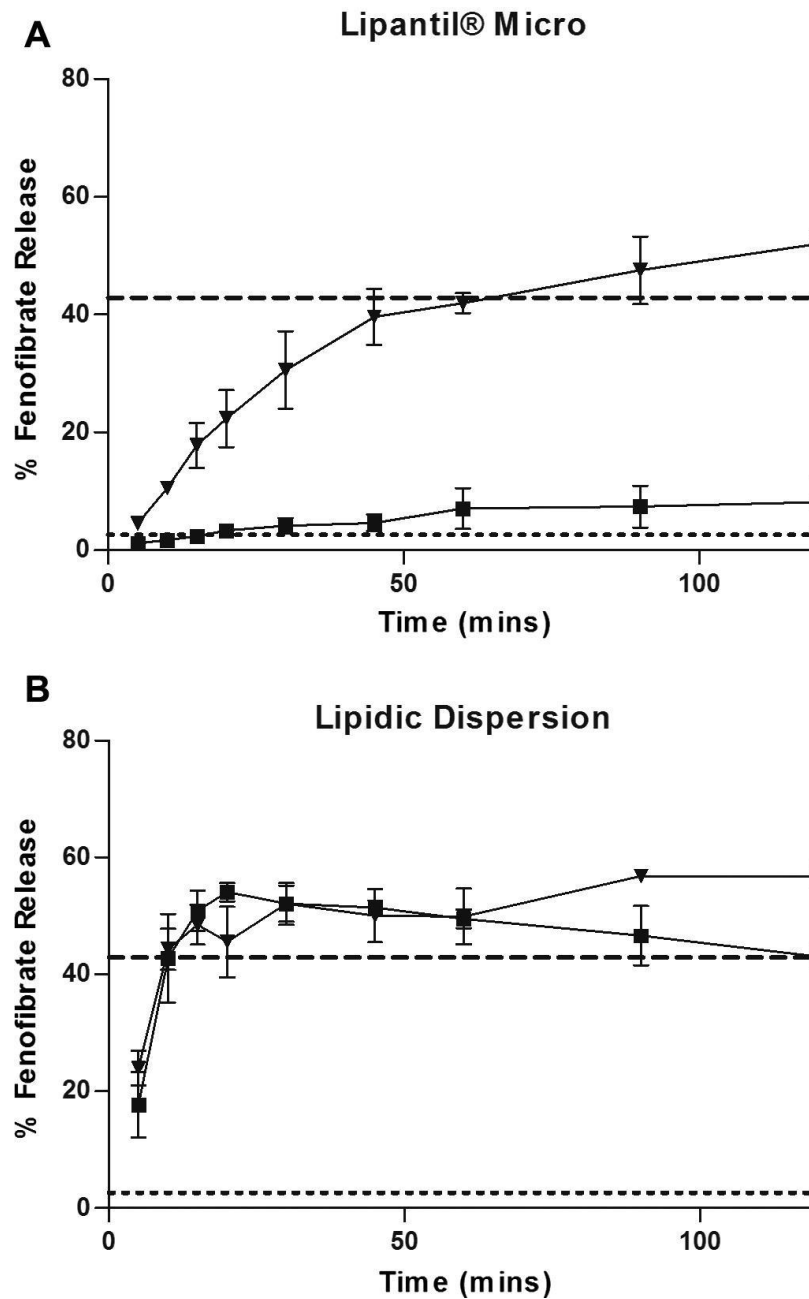

Figure 3-6 Biorelevant dissolution and solubility in simulated intestinal media, dotted line indicates fenofibrate solubility in FaSSIF-V2, dashed line indicates fenofibrate solubility in FeSSIF-V2, (■) indicates dissolution in FaSSIF-V2, (▼) indicates dissolution in FeSSIF-V2 (n = 3, mean ± SD).

Release in biorelevant media is compared to fenofibrate solubility in FaSSIF-V2 and FeSSIF-V2 in figure 3-6. The lipidic dispersion rapidly achieves concentrations greater than the measured solubility in FaSSIF-V2, through excipient mediated solubilisation.

Oral bioavailability in pigs

Figure 3-7 represents the plasma concentration profiles obtained following oral administration of 67mg of fenofibrate, as either a lipidic dispersion or Lipantil® Micro, to fasted pigs in a crossover study (mean ± SE, n = 4). Absolute bioavailability was determined relative to an intravenous control. The key pharmacokinetic parameters are summarised in table 3-1. A maximal plasma concentration of 4332 ± 1027ng/mL was observed at 1.75 ± 0.5 hr with the lipidic dispersion, while the absorption of fenofibrate from Lipantil® Micro was slower with C_{max} 2691 ± 728ng/ml at T_{max} 7.75 ± 4.92 hr. A prolonged absorption phase is evident for Lipantil® Micro, with absorption still apparent up to 12 hr post-dosing. An absolute bioavailability of 60.3 ± 8.2% was observed for the lipidic dispersion formulation and found to be not significantly different relative to absolute bioavailability of 66.1 ± 3.5% for Lipantil® Micro (mean difference = 3.482 ± 21.14%; p = 0.7634) (figure 3-8B). While there is no significant difference in the extent of oral bioavailability at 24 hr, graphical representation displays markedly different plasma concentration–time profiles. To provide further insights into the *in vivo* data, a partial AUC analysis was performed (Larsen et al., 2013). Profiles were compared up to the point where pigs were allowed access to food (0–8 hr). Partial AUC analysis demonstrates significantly greater bioavailability under the fasting period for the lipidic dispersion formulation (mean difference = 16.874 ± 7.333%; p = 0.0193) (figure 3-8A).
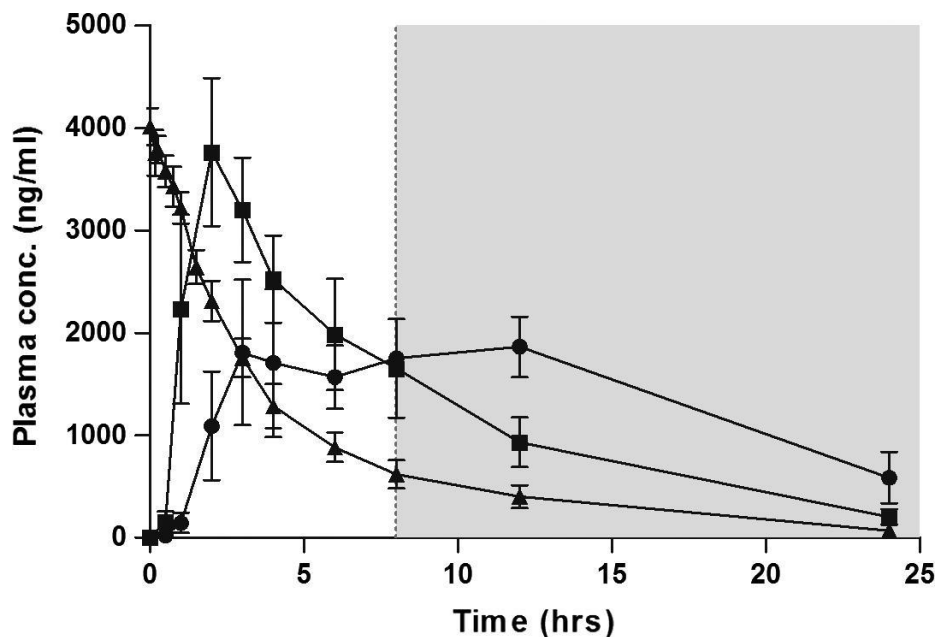
113

Figure 3-7 Plasma concentration of fenofibric acid vs. time profiles after oral administration of 67mg or i.v. administration of 25 mg fenofibrate to fasted pig (mean ± SE, n = 4), (●) indicates Lipantil® Micro, (■) indicates Lipidic Dispersion, (▲) indicates intravenous preparation. White area indicates fasting conditions, shaded area represents access to food.

Table 3-1 Summary of pharmacokinetic parameters following intravenous administration of 25mg of fenofibrate and oral administration of 67mg of fenofibrate as lipidic dispersion or Lipantil® Micro to fasted pigs (mean ± SD, n = 4).

* Bioavailability determined using $Fa_{0 \to 8hrs} = (AUC(oral)_{0 \to 8hrs}/AUC(iv)_{0 \to 24hrs})* (Dose(iv)/D(oral))$

**Bioavailability calculated using $Fa_{0 \to 24hrs} = (AUC(oral)_{0 \to 24hrs}/AUC(iv)_{0 \to 24hrs})* (Dose(iv)/D(oral))$

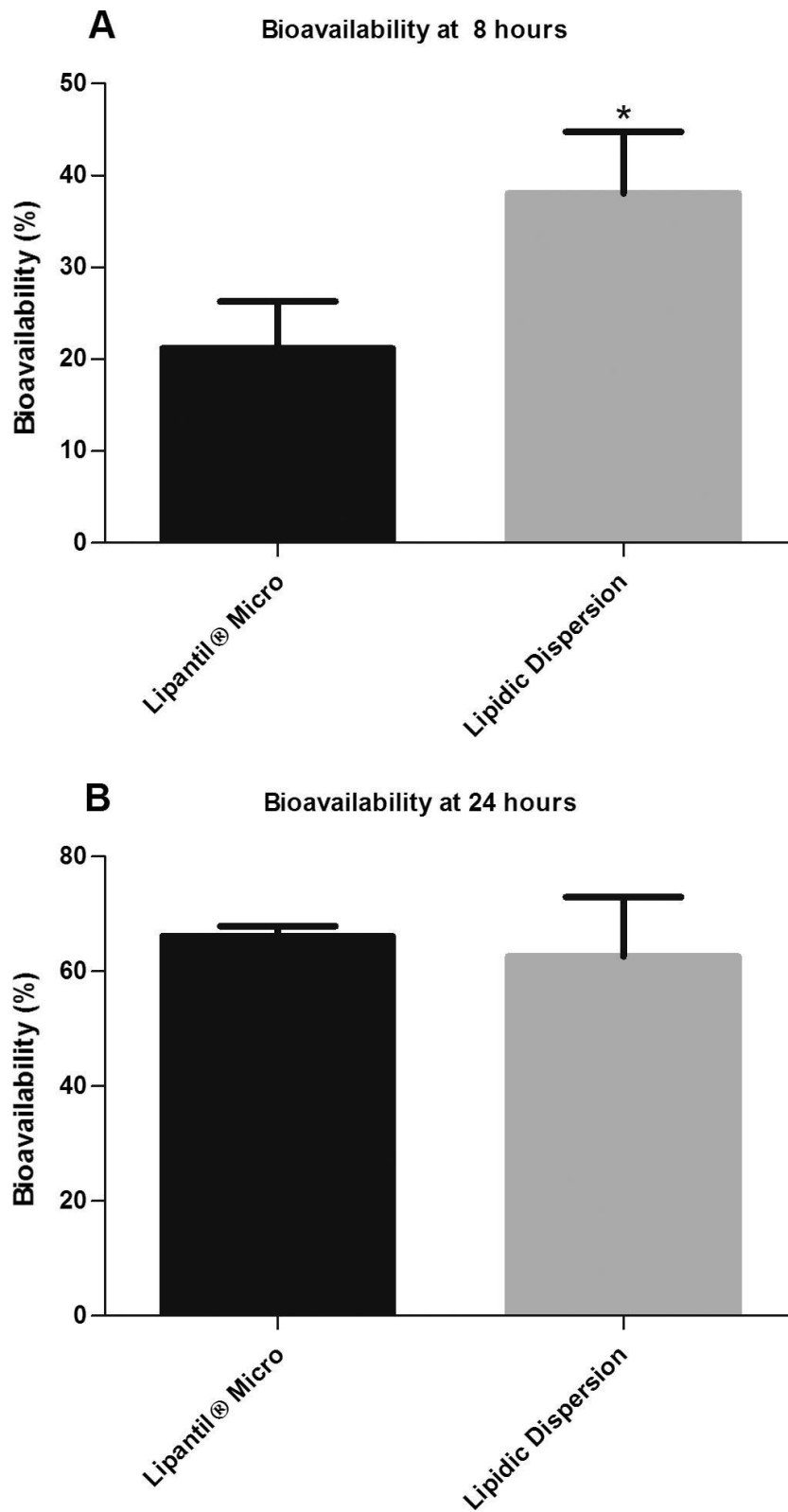| Intravenous pharmacokinetic Parameters		Oral pharmacokinetic parameters		
V_c (L/kg)	0.345 ± 0.02504		Lipidic dispersion	Lipantil® Micro
K_{el} (hr^{-1})	0.221 ± 0.064428	C_{max} (ng/mL)	4332.08 ± 1026.98	2691.35 ± 728.01
K_{el} (hr^{-1})	0.099 ± 0.038687	T_{max} (hours)	1.75 ± 0.5	7.95 ± 4.73
K_{el} (hr^{-1})	0.35125 ± 0.241289	$Fa_{0 \to 8hrs}$*	38.1% ± 13.4%	21.2% ± 10.3%
$AUC_{0 \to 24hrs}$ (ng.h/ml)	18382 ± 4591	$Fa_{0 \to 24hrs}$**	60.3% ± 8.2%	66.1% ± 3.5%

114

Figure 3-8 Bioavailability of fenofibrate from Lipantil® Micro and lipidic dispersion in fasted pigs after 8 and 24 hr (n = 4, mean ± SD).

***In silico* predictive modelling**

In silico predictive modelling was used to generate *in vitro- in vivo- in silico* correlations and predict formulation performance. These results were generated by GastroPlus™ software provided by Simulations Plus, Inc., Lancaster, California, USA. Modelling of plasma fenofibric acid concentration for the lipidic dispersion formulation, incorporating *in vitro* solubility and dissolution measurements with PKPlus™ fitted pharmacokinetic estimates resulted in an absorption model which accurately predicts the observed data in the fasted state. A highly significant positive correlation was observed between predicted and observed plasma concentration (Pearson r = 0.9616, p < 0.0001). This model predicted bioavailability in the fasted state to be 63.315% with a C_{max} of 4039.1ng/ml at 1.84 hr. Applying this model to the dissolution and solubility measurements for Lipantil® Micro formulation displays excellent correlation with the initial absorption phase, up to 8 hr (Pearson r = 0.9156, p < 0.005). However, the prolonged absorption observed *in vivo* is not modelled, with the result that T_{max} and bioavailability are lower in the predicted profile (3.76 hr and 44.8% respectively) than that seen *in vivo*.

A comparable absorption model for the fed state was utilised to examine the potential impact of formulation on fed state bioavailability. For the lipidic dispersion formulation, the fed state model showed no change in overall bioavailability with only slight changes in C_{max} (3665.7ng/ml) and T_{max} (2.08 hr), values which are still within the 80–125% bioequivalence limit. Therefore, the *in silico* model predicts that oral bioavailability for the lipid dispersion is food independent. In contrast for the Lipantil® Micro, the *in silico* model predicted a significant increase in oral

116

bioavailability in the fed state (44.8% in fasted vs. 62.8% in fed state), which is as expected for this preparation *in vivo* (Sauron et al., 2006) (figure 3-9).

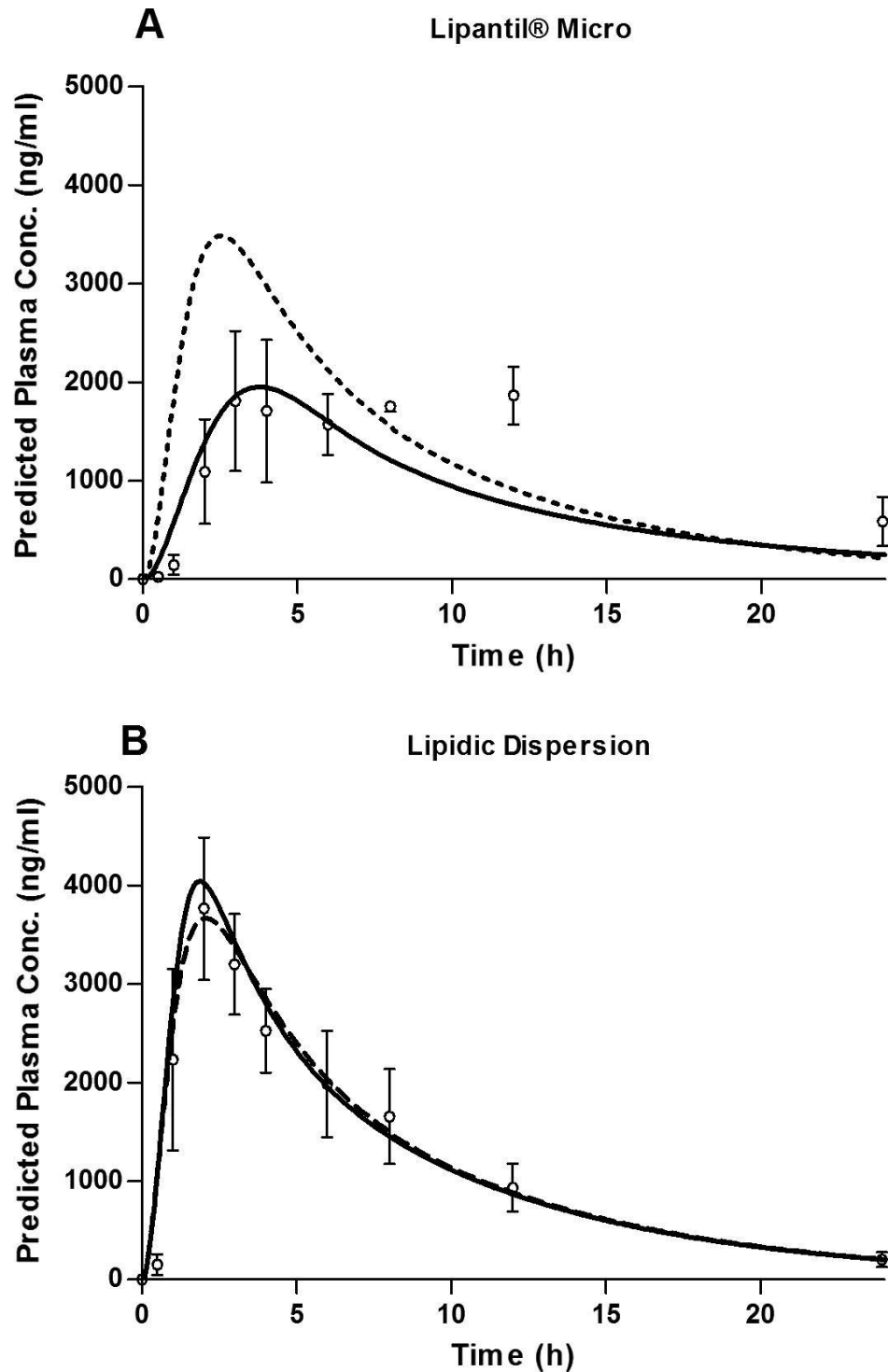*Figure 3-9 Gastroplus™ in silico model of plasma fenofibric acid concentration vs. time profile compared to observed in vivo pig data, solid line indicates predicted fasted state profile, dashed line indicates predicted fed state profile, (○) indicates observed data (n = 4, mean ± SE, reproduced from Fig. 6).*

Discussion

Fenofibrate displays poor and variable oral bioavailability mediated by its poor solubility and slow dissolution, with optimal absorption seen when co-administered with food (Sauron et al., 2006). Numerous methods have been well characterised for their ability to enhance dissolution and bioavailability of PWSD, such as fenofibrate, including nanonisation, self-emulsifying drug delivery systems (SEDDS) and solid dispersion techniques (Williams et al., 2013b, Vogt et al., 2008). In particular, nanonisation of fenofibrate has proven quite successful, resulting in a commercial preparation which has allowed dose reduction and food state independent dosing through increased bioavailability (Sauron et al., 2006, Junghanns and Muller, 2008).

This study investigates the use of solidified lipid based formulations as an alternative approach. Combination of a lipid based formulation with traditional solid dispersion technology aims to generate a novel "third-generation" solid dispersion (Vo et al., 2013). Solubilisation by ingested lipids, their digestion products and endogenous bile salts increases the bioavailability in the post-prandial state. The formulation strategy employed in this study focuses on enhancing fenofibrate bioavailability utilising this mechanism of enhanced lipid excipient mediated solubilisation, along with processing to alter the solid-state characteristics of the drug. By producing a molecularly dispersed form of the drug in a hydrophilic PVP dispersion, drug dissolution is enhanced – providing an initial 'spring' or improved dissolution rate. Physiochemical characterisation of the lipidic dispersion has demonstrated changes in the solid state of fenofibrate relative to pure drug substance and Lipantil® Micro, resulting in enhanced dissolution of fenofibrate (figures 3-4 and 3-5). Furthermore,

inclusion of lipid excipients improves solubilisation within colloidal phases that exist in intestinal media, prolonging the 'parachute' or supersaturated state. Figure 3-2 confirms the impact of lipid excipients at enhancing solubility of fenofibrate under biorelevant conditions (Williams et al., 2013b, Williams et al., 2013c).

One of the primary aims of this study was to assess the ability of this formulation to enhance bioavailability of fenofibrate in fasted pigs. A significant increase in C_{max} and shorter onset to T_{max} for the lipidic dispersion show that fenofibrate is absorbed much more rapidly and extensively in the initial absorption phase compared to Lipantil® Micro (i.e. 0–8 hr). The prolonged absorption displayed with the Lipantil® Micro formulation means no difference was observed in the overall extent of bioavailability from either formulation over the 24 hr sampling period. This may be indicative of a pronounced food effect as the secondary peak is seen 2–3 hr after the pigs were provided access to food. It is clear that absorption is still occurring at least 12 hr after the administration of Lipantil® Micro, while this is not seen for lipidic dispersion suggesting differences in the kinetics of absorption from the intestine for both preparations, which have been observed in studies where prolonged absorption or double peaking is evident (Metsugi et al., 2008).

The study also demonstrated the advantages of biorelevant dissolution testing over conventional USP testing for providing more accurate prediction *in vivo*. Using dissolution conditions as defined in the USP, the lipidic dispersion and Lipantil® Micro displayed equivalent *in vitro* release profiles, whereas under biorelevant conditions, distinct differences between the formulations were observed. In this case, biorelevant dissolution testing has provided a qualitatively accurate prediction of *in*

vivo performance, with rapid and extensive dissolution in FaSSIF-V2 predicting the increased rate of bioavailability for the lipidic dispersion, whereas slow dissolution of Lipantil® Micro *in vitro* is reflected in its slower absorption *in vivo*. Enhanced solubilisation caused by incorporation of lipidic excipients results in rapid achievement of concentrations greater than thermodynamic solubility, which are maintained throughout the test. By overcoming the solubility limitation of fenofibrate in FaSSIF-V2, through inclusion of lipidic excipients, there is no enhancement in dissolution in the fed state compared to the fasted state, as is seen with Lipantil® Micro. It appears that dissolution in the fasted state medium has been maximised indicating potential of the lipidic dispersion to eliminate any food effects on dissolution and subsequent bioavailability of fenofibrate.

The solubility of fenofibrate in the biorelevant media with added lipid excipients was used to predict the *in vivo* solubility at the site of absorption. However, this approach does not make allowances for the impact of digestion of lipid excipients on drug solubilisation *in vivo*. In the case of fenofibrate, previous studies have shown that *in vitro* digestion of lipid based formulations containing similar long chain lipids to the current lipidic dispersion, had a limited impact on drug solubility post-digestion. Furthermore, it appears that in the case of the non-ionisable compounds, such as fenofibrate, the risk of reduced *in vivo* absorption due to digestion-induced drug precipitation is low (Stillhart et al., 2014b, Griffin et al., 2014, Thomas et al., 2014). However, in the case of weak bases, in particular, there is potential for formulation digestion by intestinal lipases to result in a loss of solubilisation and cause precipitation (Christophersen et al., 2014, Stillhart et al., 2014a). These effects

appear to be drug and formulation specific and currently need to be assessed on a case by case basis (Williams et al., 2014).

Incorporation of *in vitro* measurements with i.v. pharmacokinetics through *in silico* modelling can be used to accurately predict formulation performance through the generation of *in vitro- in vivo- in silico* correlations (Kostewicz et al., 2014a). The resultant model predicts a profile which accurately reflects *in vivo* performance for the lipidic dispersion. The rapid dissolution and increased solubilisation measured *in vitro* are predicted to maximise absorption in the fasted state, with the corresponding fed state model predicting no increase in dissolution, solubilisation or absorption. The prolonged absorption of Lipantil® Micro proved difficult to model with only a weak overall correlation, though this correlation is much stronger in the initial absorption phase. While this model requires further validation, initial estimates demonstrate that there is potential for the lipidic dispersion formulation to eliminate food effect by enhancing solubility and dissolution in the fasted state to levels comparable to the fed state, where optimal absorption of fenofibrate is observed.

For Lipantil® Micro, it is clear from the *in vivo* profile that there is prolonged absorption and/or a secondary absorption phase occurring. Possible explanations suggested included either prolonged drug residence in the gastrointestinal tract or significant post-prandial effects, or potentially a combination of both these factors. To explore the validity of these hypotheses further the *in silico* model was modified by introducing (i) feeding at 8 hr post-dose; (ii) reduced gastrointestinal transit or (iii) a combination of reduced transit and feeding at 8 hr. Introducing a feeding stage 8

hr post-dose did not result in a change in the predicted profile. In the case of a reduced gastrointestinal transit, as can be seen from the profiles generated in figure 3-10, prolonged transit results in slower absorption. Finally, coupling prolonged transit with feeding at 8 hr predicted a secondary peak in plasma profiles, which improved the correlation with the *in vivo* data (r = 0.8997, p ⩽ 0.001). To achieve this result the gastrointestinal transit time was increased to approximately 10 hr, but these values are speculative rather than predictive. The improved correlation obtained using *in silico* modelling therefore suggests that a combined effect of prolonged drug residence in the intestine coupled with a post-prandial intestinal conditions at 8 hr post-dose, may be used to explain the secondary absorption phase observed for the Lipantil® Micro formulations. However, further work is required to assess the accuracy and validity of these predicted profiles.
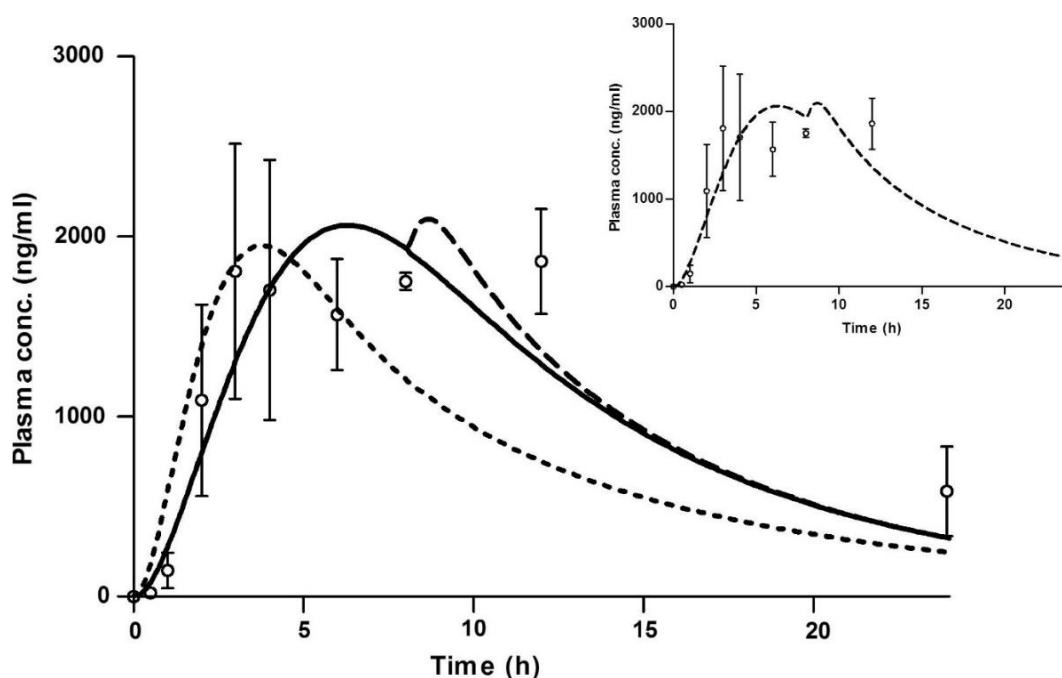
Figure 3-10 Gastroplus™ in silico model of plasma fenofibric acid concentration vs. time profile compared to observed in vivo pig data, dotted line indicates physiological model in fasted state (reproduced from Fig. 3-9), solid line indicates predicted fasted state profile with delayed small intestinal transit, dashed line indicates predicted fasted state profile with delayed small intestinal transit and a feeding step at 8 hr (data also inset), (○) indicates observed data (n = 4, mean ± SE, reproduced from Fig. 3-7).

Conclusions

The novel lipidic dispersion demonstrates improved biorelevant solubility and dissolution of fenofibrate relative to Lipantil® Micro, eliminating fed state enhancement of dissolution. The lipidic dispersion increased the rate of bioavailability of fenofibrate in fasted pigs, while the extent absorption was similar to Lipantil® Micro. Biorelevant dissolution testing provides a qualitatively accurate prediction of *in vivo* formulation performance. This approach appears to be appropriate for non-ionisable drugs, such as fenofibrate. Drug and formulation specific characterisation is still required to assess formulation performance. Combination of *in vitro* solubility and dissolution measurements with *in vivo* pharmacokinetic measurements to produce *in silico* generated simulated profiles can accurately predict *in vivo* profiles and has been used to predict a reduction in food effects on oral fenofibrate bioavailability.

Chapter 4 : Assessing absolute oral bioavailability of celecoxib in pigs

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Introduction

Poorly water-soluble drugs continue to pose significant challenges for oral drug delivery. With the ever increasing prevalence of lipophilic, poorly soluble compounds in drug development pipelines, the identification of compounds with optimized pharmacodynamic properties, but poor 'developability' owing to sub-optimal absorption properties leads to formulation and delivery challenges in drug development, where significant delays or even failure to gain approval can occur (Butler and Dressman, 2010, Hauss, 2007). The successful delivery of these challenging compounds will often rely on the use of novel bioenabling formulations, designed to enhance their *in vivo* solubility and/or dissolution (Williams et al., 2013b). Dissolution is considered the rate-determining step for absorption of poorly soluble and highly permeable compounds, and bioenabling formulations are frequently investigated for their potential to improve oral bioavailability through increases in the rate of absorption and in overall extent of bioavailability (Buckley et al., 2013). Development of novel bioenabling formulations relies heavily on the ability to screen performance pre-clinically, and requires suitable marker compounds and reliable *in vitro* and *in vivo* models to predict the likely effects in clinical use.

Previous studies in the current thesis have focused on the utility of fenofibrate as a model compound in the assessment of bioenabling formulations. Based on these studies, a second model drug was desired to act as a marker of both bioenabling approaches and of food dependent bioavailability, particularly with a food effect which is less formulation dependent than that of fenofibrate, and which can act as a 'borderline' case study with a low to moderate food effect. Celecoxib was identified

125

as such a candidate. As a pre-cursor to any studies assessing bioavailability altering approaches, it is desirable to first assess absolute bioavailability *in vivo*. The aim of the current study is, therefore, to assess the absolute bioavailability of celecoxib, and its potential to act as such a marker compound in biorelevant *in vitro* screening and in an *in vivo* fasted pig model for assessment of bioenabling approaches.

Celecoxib is a non-steroidal anti-inflammatory drug (NSAID) which exerts its pharmacological action by selective inhibition of the cyclooxygenase-2 (COX-2) isozyme. It is widely used in the treatment of osteo- and rheumatoid arthritis and ankylosing spondylitis (Shi and Klotz, 2008, Davies et al., 2000). Celecoxib is highly lipophilic and very poorly water soluble, with an approximate aqueous solubility of 1µg/ml, but demonstrates good permeability and is classed as a BCS class II compound with dissolution/solubility limited oral absorption (Guzman et al., 2007, Paulson et al., 2001, Laine et al., 2016). As a result, the marketed Celebrex™ formulation was designed to maximise dissolution, with particle size identified as a critical quality attribute (CQA) during the regulatory process. The commercial preparation has a D_{90} below 25µm and contains sodium lauryl sulphate as a wetting agent, with the function of improving dissolution of the API *in vivo* (Laine et al., 2016, FDA, 1998). As a BCS class II compound, a significant positive food effect is anticipated for celecoxib. However, while a significant increase in C_{max} (1.9 fold increase) is observed in the fed state, mediated by increases in post-prandial solubilisation, there is only a modest increase in overall bioavailability (approximately 1.1 to 1.3 fold), allowing Celebrex™ to be dosed independent of prandial state (Paulson et al., 2001, Pfizer Inc., 2000, Lyng et al., 2016).

Due to its poor solubility/dissolution celecoxib has been repeatedly used as a model compound for assessment of bioenabling approaches, including salt formation (Guzman et al., 2007), microcrystals (Nasr, 2013), lipid-based formulations (Song et al., 2014, Nguyen et al., 2013, Subramanian et al., 2004), nanoparticle formation (Morgen et al., 2012, Liu et al., 2010), cyclodextrin based formulations (Rawat and Jain, 2004) and a mesoporous silica based amorphous dispersion (Laine et al., 2016). The effect of food on celecoxib has also been assessed in both the pre-clinical animal models and in humans, with a moderate increase in bioavailability observed (Gong et al., 2012, Davies et al., 2000, Lyng et al., 2016, Paulson et al., 2001, Shono et al., 2009).

To date the majority of pre-clinical, *in vivo* studies of celecoxib bioavailability and assessment of bioenabling formulations, as with other compounds, have been performed in rats and/or dogs. While there is a well-established history of using these pre-clinical animal models, there is also significant limitations in their ability to predict human *in vivo* performance (Musther et al., 2014, Hatton et al., 2015, Sjogren et al., 2014). In this regard, the use of the pig model in pre-clinical assessment has expanded significantly in recent years (Colleton et al., 2016). The pig presents numerous advantages in the assessment of pre-clinical formulations, particularly with regard to the anatomical and physiological similarities in the gastrointestinal tract of pigs and humans (Hatton et al., 2015, Sjogren et al., 2014, Swindle and Smith, 1998, Suenderhauf and Parrott, 2013). While there are many similarities in the gastrointestinal structure and physiology between pigs and humans, there are also significant differences, particularly with regard to metabolising enzymes (Henze et

al., 2018b). Overall, while "animal models remain a poor simulacrum of human physiology", they are still widely utilised to anticipate human pharmacokinetics and to discriminate product performance (Hatton et al., 2015). Recognising the limitations of a "one size fits all" approach in the context of animal modelling, and the absence of one ideal species that mimics closely human GI physiology and function, the most reliable approach appears to be the utilisation of numerous different animal models to model the various aspects of human pharmacokinetics (Hatton et al., 2015).

With this in mind, the aim of the current study was to determine the bioavailability and pharmacokinetics of celecoxib in fasted pigs in order to assess its utility as a model compound for the assessment of bioenabling formulations and to compare bioavailability in pigs to that previously determined in the dog model (Paulson et al., 2001). Given the moderate food effect observed for the Celebrex™ formulation, celecoxib may also be appropriate as a marker compound for development of a food-effect model in fasted pigs, acting as a 'borderline' case study assessing the sensitivity of such model.

The aims of the current study were, therefore, to;

- Characterise the biorelevant *in vitro* performance of celecoxib, with regard to solubility and dissolution, in order to predict likely *in vivo* performance

- Develop a suitable intravenous (i.v.) formulation to allow for determination of absolute bioavailability

- Assessment of celecoxib bioavailability and pharmacokinetics using Celebrex™ and the in-house developed i.v. formulation

128

These studies were performed as a partially randomised three-way crossover using male Landrace pigs where animals were dosed two oral preparations and an i.v. formulation in the fasted state with blood sampling performed over 24 hours. The third leg of the study involved an investigational formulation of celecoxib. However due to confidentiality considerations with the industrial partner providing the investigational formulation, the oral pharmacokinetic data is unavailable for publishing. The data presented relate solely to the commercial Celebrex™ formulation and an in-house developed intravenous (i.v.) preparation.

Materials and methods

Materials

Celecoxib API was purchased from Kemprotec Ltd. (UK) and celecoxib reference standard was received from Institut für Pharmazeutische Technologie, Goethe Universität, Frankfurt am Main, Germany. 2, 5 – di methyl celecoxib, polyethylene glycol (MW400) acetonitrile, sodium chloride and ethyl acetate were purchased from Sigma-Aldrich (Ireland). Sterile 0.9% sodium chloride solution was purchased from B. Braun Medical Ltd. (Ireland). Celebrex™ capsules were obtained from Institut für Pharmazeutische Technologie, Goethe Universität. All other chemicals and solvents were of analytical grade or HPLC grade respectively and were purchased from Sigma-Aldrich (Ireland).

Celecoxib HPLC analysis

Quantification of celecoxib was carried out using HPLC using a method developed based on the methods of Schonberger *et al*. and Tan *et al*. (Schonberger et al., 2002, Tan et al., 2009). The HPLC system consisted of an Agilent 1120 compact LC system equipped with auto-sampler and variable wavelength detector set at UV 254nm. The mobile phase was a mixture of acetonitrile and water (60% and 40% respectively) eluted at 1 ml/min through a Licrosphere C_{18} 5µM RP-Select-B column (250 x 4.6 mm) equipped with a C_{18} RP 4 x 3mm guard cartridge (Phenomenex, Macclesfield, UK). Retention time of celecoxib was approximately 8.75 minutes.

Biorelevant solubility and dissolution

FaSSIF-V2 and FeSSIF-V2 were prepared as outlined in the literature (Jantratid et al., 2008).

Solubility studies were carried out by the addition of excess celecoxib to biorelevant media and using a standardised shake flask method with a shake time 24 hr at 37°C (Juenemann et al., 2011). 2ml samples were removed at 24 hr and added to 2ml centrifuge tubes. Samples were centrifuged at 16,500g for 13 min (Hermle z233M-2 fixed angle rotor centrifuge, HERMLE Labortechnik GmbH, Wehingen, Germany). The resultant supernatant was free from particles and was removed and centrifuged again under similar conditions. The resulting supernatant was analysed using HPLC after appropriate dilution with acetonitrile.

Biorelevant dissolution studies were carried out in triplicate with an Erweka DT600 dissolution test system (Erweka GmbH, Germany). Tests were performed in 500ml FaSSIF-V2 or FeSSIF-V2 at 37 ± 0.5°C using USP type II paddle method at 75rpm. Celebrex™ 100mg capsules were placed in the dissolution vessel with wire sinkers. Samples of 4ml were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes, immediately followed by the addition of an equal volume of fresh, pre-warmed medium. The withdrawn samples were filtered through a 0.45µm regenerated cellulose filter (Whatman® Roby 25/0.45 RC-GF, GE Healthcare Life Sciences, Buckinghamshire, UK), discarding the first 2ml. The resultant filtrate was visually assessed as being clear and free from particles. The sample was immediately diluted with an appropriate volume of acetonitrile and analysed using the HPLC method outlined above.

Plasma Extraction

Extraction of celecoxib from plasma was developed based on a method adapted from that of Schonberger *et al*. (Schonberger et al., 2002). 0.5mL was spiked with 50μL of 2, 5-dimethyl celecoxib (10μg/ml) as an internal standard. Proteins were precipitated by adding 1ml of acetonitrile and 0.5ml of saturated sodium chloride. Samples were then centrifuged at 11,500g for 9 min (Hermle z233M-2 fixed angle rotor centrifuge, HERMLE Labortechnik GmbH, Wehingen, Germany) and supernatant was removed and added to a 15ml tube. 4ml of ethyl acetate was added and extraction was carried out with shaking for 10 minutes. The sample was centrifuged at 3220g for 4 minutes (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany). Supernatant was transferred to a 5ml tube and evaporated to dryness under N_2 at 50°C. A second similar extraction step was carried out and supernatant was added to corresponding 5ml tube from step 1. The residue was reconstituted with 200μL of mobile phase followed by vortexing for 1 minute.

Samples were analysed using HPLC as per method described above with the addition of a cleaning step at the end of each run where a mobile phase consisting of 85% acetonitrile and 15% water was passed through the column for 5 minutes before re-equilibrating the column with 60% acetonitrile and 40% water for a further 5 minutes. The retention times of celecoxib and internal standard were approximately 8.75 and 10.2 minutes respectively.

Plasma standards were prepared by spiking 0.5mL of blank plasma with 50μL of appropriate concentration of celecoxib reference standard dissolved in acetonitrile. Plasma concentrations were determined by comparing celecoxib peak area to internal standard peak area.

132

Intravenous preparation

An i.v. formulation was prepared based on a modification of a formulation used by Paulson *et al.* and Guzman *et al.* (Paulson et al., 2001, Guzman et al., 2007). Celecoxib solubility in varying concentrations of PEG-400 and ethanol mixed with normal saline was first determined.

When a suitable solvent system was identified a formulation for i.v. administration was prepared by dissolving 0.5g of celecoxib in 10ml of solvent (60:20:20 PEG 400/Ethanol/Saline). This preparation was then filtered using a 0.20μm PES membrane filter (Filtropur S 0.2, Sarstedt AG & Co., Nümbrecht, Germany) under aseptic conditions into a sterile vial and sealed. The final solvent system consisted of 60% PEG 400, 20% ethanol and 20% physiological saline. The final formulation was assayed for celecoxib content by HPLC after appropriate dilution with acetonitrile to ensure no loss of drug on filtration.

Oral bioavailability in pigs

The study was carried out under licences issued by the Department of Health, Ireland as directed by the Cruelty to Animals Act, Ireland and EU Statutory Instruments. Local University ethical committee approval was also obtained. The study was a partially randomised three-way crossover design, where the pigs were randomly allocated to one of the two oral formulations on the first leg, followed by a crossover for the second leg. The final leg of the study involved an intravenous study on all pigs.

Six male Landrace pigs (16.8–18.5kg, mean 17.5kg) were sourced locally and housed at the University's Biological Services Unit. Pigs were fasted for 16 hours before experiments. On day 1, an indwelling intravenous catheter was inserted into the

133

jugular vein, under general anaesthesia as previously described (Griffin et al., 2014). Following an overnight fast on day 3, oral formulations were administered. Celebrex™ was delivered in its commercial capsule with the aid of a dosing gun after which the pigs received 50 mL of water via syringe. After dosing, pigs were returned to their pens. Blood samples (4mL) were collected at time zero (pre-dosing) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12 and 24 hours post dosing. Water was available *ad libitum* throughout the study period and the animals were fed 8 hours post dose.

For the intravenous treatment, animals were administered 100mg celecoxib by slow intravenous bolus injection of 2mls of a solution containing 50mg/ml fenofibrate in 60% w/w PEG-400, 20% w/w ethanol and 20% w/w physiological saline into an ear vein. Blood sampling was performed as outlined above, with additional blood samples taken at 0.25 and 0.75hr post dose. All blood samples were collected in heparinised tubes (Sarstedt AG & Co, Germany) and immediately centrifuged at 3220*g* for 5 minutes at 4°C (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany). Plasma was collected and stored at −80°C prior to analysis.

A seven day washout period was observed between each leg of the study. One animal was lost to oral dosing due to loss of patency in the indwelling intravenous catheter over the course of the study. Another two animals were excluded from the intravenous leg of the study due to difficulties in cannulation of the ear vein for i.v. administration.

Pharmacokinetic Data Analysis

Intravenous pharmacokinetic parameters were fitted to a one compartment model using the PKPlus module in Gastroplus™ (version 8.5, Simulations Plus, Lancaster, CA). The AUC for celecoxib was calculated using Prism (version 5, GraphPad Software Inc., La Jolla, Ca.). The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs. time profiles. The Absolute Bioavailability (Fa) was calculated according to equation 4-1 below:

$$Fa = \left(\frac{AUC_{oral}}{AUC_{i.v.}} \right) \cdot \left(\frac{Dose_{i.v.}}{Dose_{oral}} \right) \qquad (4\text{-}1)$$

As i.v. data was only available for 3 animals the bioavailability was calculated by comparing the oral AUC for each animal to the mean i.v. AUC.

All pharmacokinetic parameters are reported as mean ± SD, with the exception of T_{max}, which is reported as median (range).

135

Results

Validation of assay for quantification of celecoxib concentrations in plasma

To ensure accurate and reproducible quantification of celecoxib from plasma both the assay and extraction procedure were validated. The limit of quantification for the *in vitro* assay was 20ng/ml and the method was linear in the range 20-2000 ng/ml. Calibration curves were considered linear when $r^2>0.99$. Figure 4-1 contains a sample standard chromatogram, containing plasma spiked with both celecoxib and internal standard. Figure 4-1A contains the complete chromatogram, incorporating the wash step, while figure 4-1B contains an exploded view of the region of interest. Table 4-1 contains analysis of intra- and inter-day assay variation form assay validation. In all cases variation was <5% confirming assay reproducibility.

Concentration (ng/ml)	Intra-day AUC			Inter-day AUC		
	Mean	Std. Dev.	% RSD	Mean	Std. Dev.	% RSD
2000	1007328	6971	0.69	1029572	31105	3.02
1000	497703	2065	0.41	501178	5767	1.15
500	199547	887	0.44	201419	966	0.48
200	103024	829	0.80	103744	727	0.70
100	51153	971	1.90	50698	281	0.55
50	22903	388	1.69	22808	736	3.22
20	11400	373	3.27	11322	349	3.08

Table 4-1 Intra- and inter-day celecoxib assay validation (n=3)

Table 4-2 displays data detailing recovery of both celecoxib and internal standard from each spiked plasma sample. In all cases recovery of celecoxib is >75%, while recovery of internal standard is >84%. The high recovery figures, coupled with low variation (standard deviation of 8% and 5% respectively) indicate a reliable and reproducible recovery. This was also demonstrated from the similarity in slope of the

136

between the lines of best fit for both calibration of *in vitro* standards (m = 509.12)

and plasma standards (m = 472.26) with a difference of approximately 7%. The LOQ
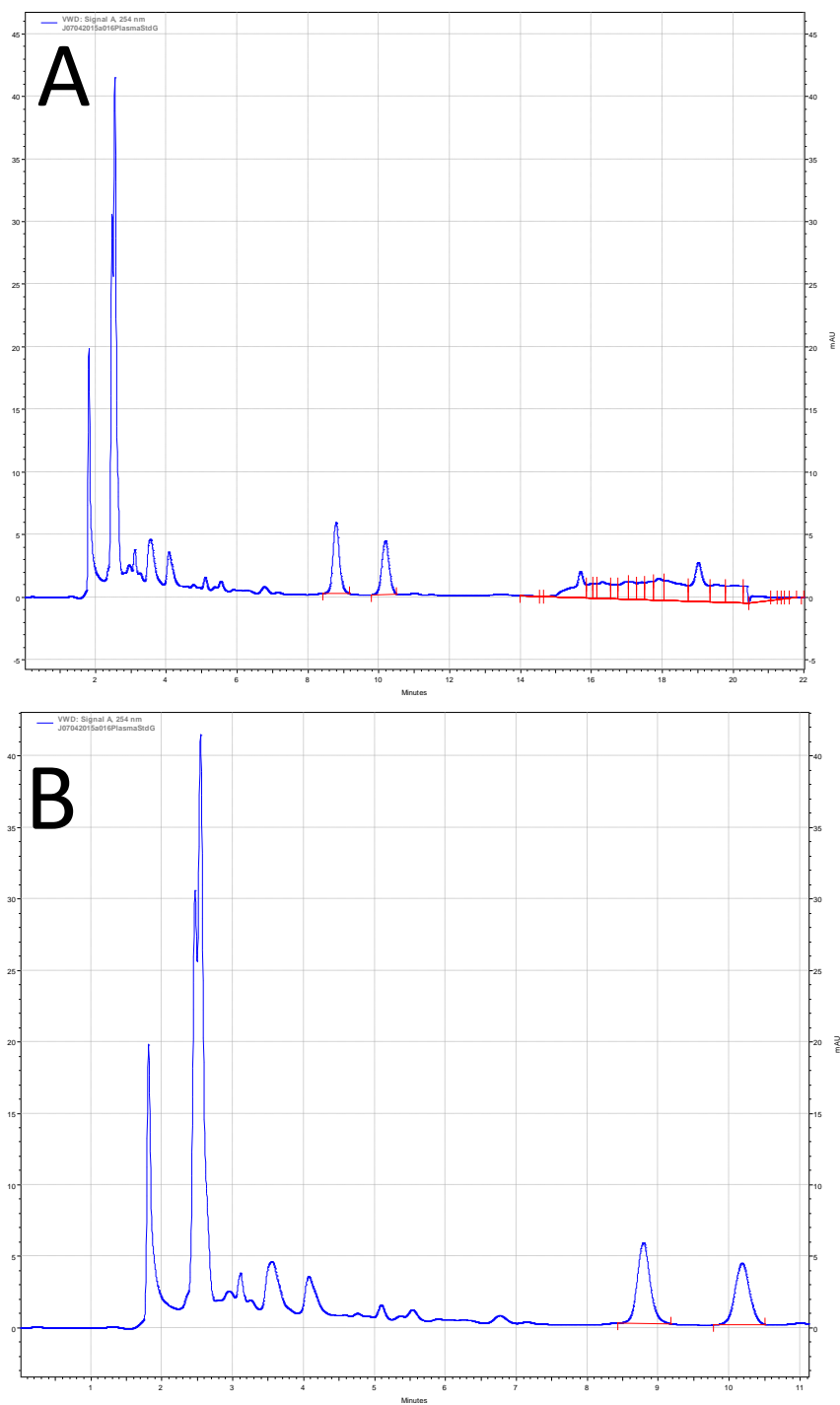
for plasma assay was 50ng/ml.

Figure 4-1 Sample celecoxib standard chromatogram containing blank plasma spiked with celecoxib and internal standard. (A) complete chromatogram incorporating wash step. (B) Exploded view

Concentration (ng/ml)	Celecoxib Recovery	Internal Standard Recovery
2000	0.943485311	0.961689
1000	0.783124062	0.845516
500	0.834282651	0.857017
200	0.949426133	0.934266
100	0.838927685	0.919651
50	0.752158015	0.956941
Average	0.850234	0.912513
Std. Dev.	0.081289	0.049969

Table 4-2 Recovery of celecoxib and internal standard from plasma standards

Figure 4-2 displays data above standardised to recovery of internal standard for each sample by plotting the peak area ratio of celecoxib: internal standard as a function of concentration. This adjustment results in comparable calibration for both *in vitro* and plasma standards indicating that the peak area ratio of celecoxib: internal standard is an appropriate method to quantify plasma celecoxib concentrations from *in vivo* samples.
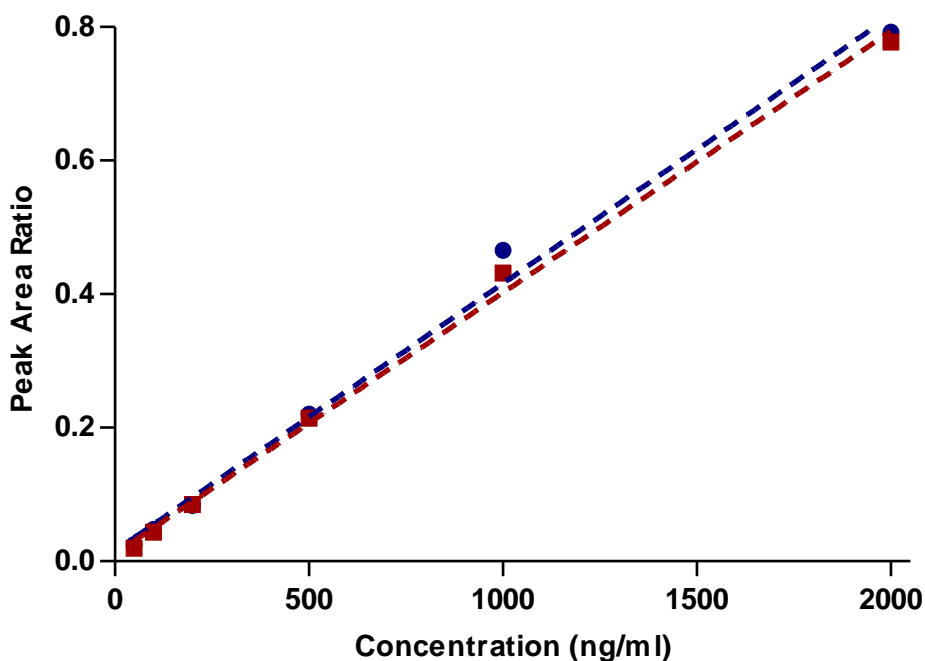

Figure 4-2 Celecoxib plasma extraction calibration (red line); comparison to in vitro standards (blue line); standardised for internal standard recovery

Biorelevant solubility and dissolution

Solubility of celecoxib in biorelevant media was measured in order to assess the solubility at the site of absorption, allowing prediction of the likely limiting factor to oral absorption (Butler and Dressman, 2010, Augustijns et al., 2014). Celecoxib solubility was low in a fasted state representative medium (FaSSIF-V2) with an observed solubility of 8.638 ± 0.275 μg/ml, and increased to 99.076 ± 10.293 μg/ml in the fed state medium (FeSSIF-V2) (figure 4-3). The FeSSIF-V2/FaSSIF-V2 ratio demonstrates an 11.46 fold increase in solubility appears to be relatively comparable to previous studies examining the fed: fasted solubility ratio for celecoxib (Shono et al., 2009).

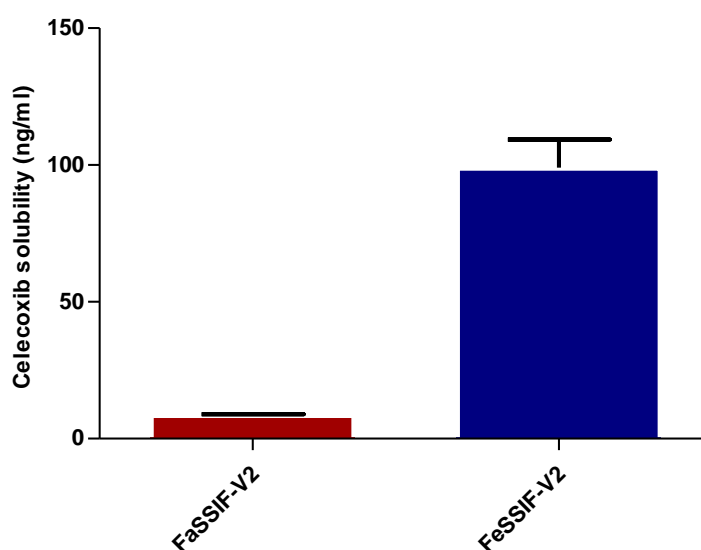*Figure 4-3 Biorelevant solubility of celecoxib in simulated intestinal media (mean ± SD, n=3)*

Subsequent to assessment of celecoxib biorelevant solubility, the dissolution of Celebrex™ in biorelevant media was evaluated in USP type II dissolution apparatus with 500ml of biorelevant media, in both FaSSIF-V2 and FeSSIF-V2 (figure 4-4). Dissolution from the Celebrex™ formulation appears to occur quite rapidly, reaching

plateau concentrations within 10 minutes for the fasted state medium and within 30 minutes for the fed state medium. Moreover, the plateau concentrations reached reflect well the biorelevant solubility measurements observed for celecoxib in both the fasted and fed state media, with the kinetic solubility in FaSSIF-V2 only slightly greater than that previously determined (figures 4-3 and 4-4). However, it is worth noting that dissolution in both the fasted and fed state is incomplete with approximately 50% of the dose dissolved in the fed state, and only 6-7% of the dose dissolved in fasted state. These observations, coupled with the rapid dissolution, suggest solubility, rather than dissolution rate, hinders celecoxib absorption *in vivo*.
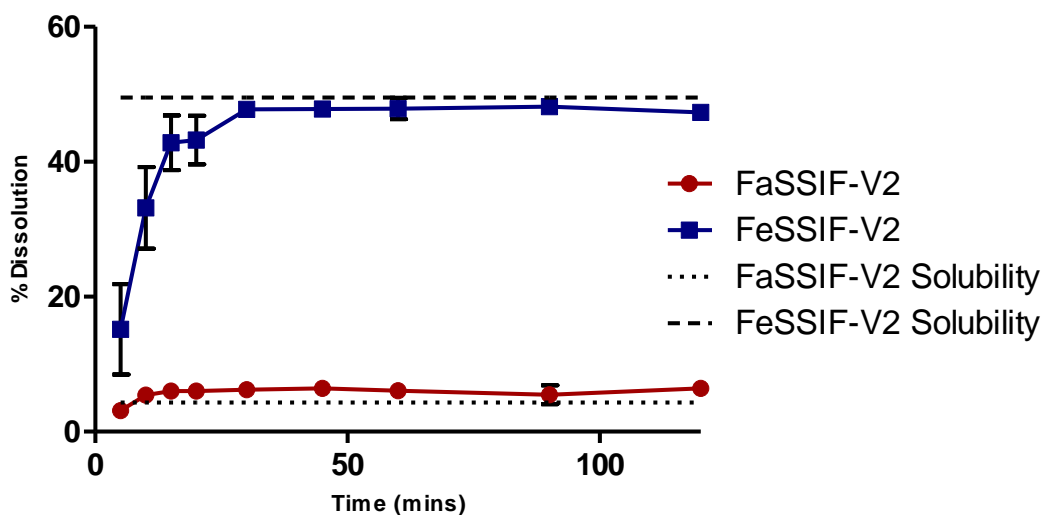

Figure 4-4 Biorelevant solubility of celecoxib and dissolution of Celebrex™ in simulated intestinal media (n = 3, mean ± SD)

Development of intravenous formulation of celecoxib

In order to assess the absolute bioavailability and total extent of systemic absorption of celecoxib, an intravenous formulation for delivery of 100mg celecoxib was required. However, no clinically approved i.v. celecoxib formulation is available and this necessitated design of a suitable intravenous formulation. The intravenous vehicle used in the only other comparable studies of absolute bioavailability did not provide sufficient solubility to dissolve 100mg of celecoxib in an appropriate volume for administration (Guzman et al., 2007, Paulson et al., 2001). This necessitated use of a co-solvent parenteral vehicle.

A formulation for intravenous administration was prepared based on equilibrium solubility in a three component solvent system consisting of PEG-400, ethanol and physiological (0.9%) saline using the following considerations;

i) Ethanol concentration no greater than 20% based on previous in-house experience.

ii) PEG-400 concentration no greater than 66% due to viscosity issues in administration.

iii) Dose volume of no more than 3ml due to restrictions in administration in ear vein; ideal volume was considered 2ml.

iv) Preference for 100mg dose to allow direct comparison between oral and i.v. formulation.

Equilibrium solubility in a range of three-component solvent systems was determined and results are summarised in figure 4-5 below. Vehicle C was chosen based on having sufficient solubility for a 100mg dose in 2ml (50mg/ml;

141

approximately 62% loading). The celecoxib content of the i.v. formulation was assayed post filtration to ensure dose delivered was accurate. Analysis displayed that the mean 2ml dose was 94.31 ± 1.72mg.
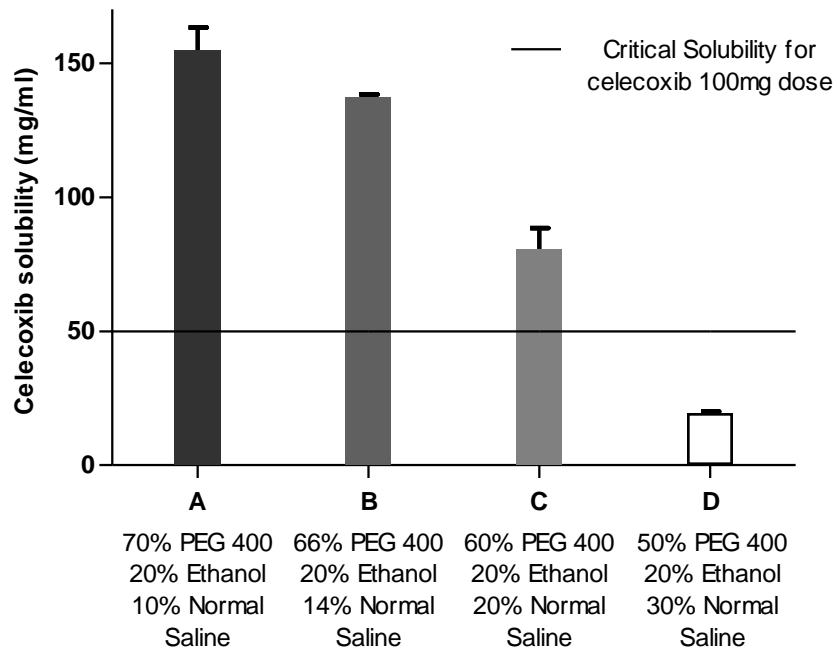
Figure 4-5 Celecoxib equilibrium solubility in i.v. formulation media, Mean ± SD (n=3)

Oral pharmacokinetics in pigs

Figure 4-6 Mean celecoxib plasma concentration profile after oral administration of 100mg celecoxib as Celebrex™ Hard Capsules to fasted pigs (mean ± SEM, n=5)

Figure 4-6 displays the mean plasma concentrations versus time profiles of after oral dosing of Celebrex™ to male landrace pigs in the fasted state. Absorption appears to be relatively rapid. With a median T_{max} of 2.5 hours (range 2.5-5) and elimination is virtually complete at 12 hours, with negligible or non-quantifiable concentrations observed at 24 hours for all pigs. The maximal plasma concentrations (C_{max}) and the time they occurred (T_{max}) were observed directly from each individual profile and are summarised in table 4-3.

Intravenous pharmacokinetics and absolute bioavailability of celecoxib in pigs

Figure 4-7 Mean celecoxib plasma concentration profile on linear (A) and log transformed (B) scales after intravenous administration of 100mg celecoxib to fasted pigs (mean ± SEM, n = 3)

Figure 4-7 displays the mean plasma concentration in linear (A) and log transformed (B) plots for the pigs dosed with the intravenous formulation graphed against time. The log transformed i.v. data display excellent linearity ($r^2>0.9$) indicating the *in vivo* data can be described using one compartment. The area under the curve (AUC) of the mean i.v. plasma profile was used to calculate the absolute bioavailability of celecoxib in the oral study leg using equation 4-1. Table 4-3 summarises these pharmacokinetic measurements from the oral and i.v. profiles.

Intravenous Pharmacokinetic Parameters		Oral Pharmacokinetic Parameters	
Vd_{ss} (L/kg)	0.7359 ± 0.095	**Celebrex™**	
K_{el} (hr^{-1})	0.3878 ± 0.0697	C_{max} (ng/ml)	3726.3 ± 2335.2
Clearance (ml/min/kg)	4.743 ± 0.974	T_{max}* (hours)	2.5 (2.5-5)
$AUC_{0 \to 24hrs}$ (ng.h/ml)	18536 ± 3105	$AUC_{0 \to 24hrs}$ (ng.h/ml)	16539 ± 10274
$Fa_{0 \to 24hrs}$	100%	$Fa_{0 \to 24hrs}$	$89.23\% \pm 55.43\%$

*Table 4-3 Pharmacokinetic parameters after intravenous administration of 100mg of celecoxib (mean ± SD, n=3) and oral administration of 100mg of celecoxib as Celebrex™ to fasted pigs (mean ± SD, n=5), *(median (range))*

Individual pig plasma concentrations

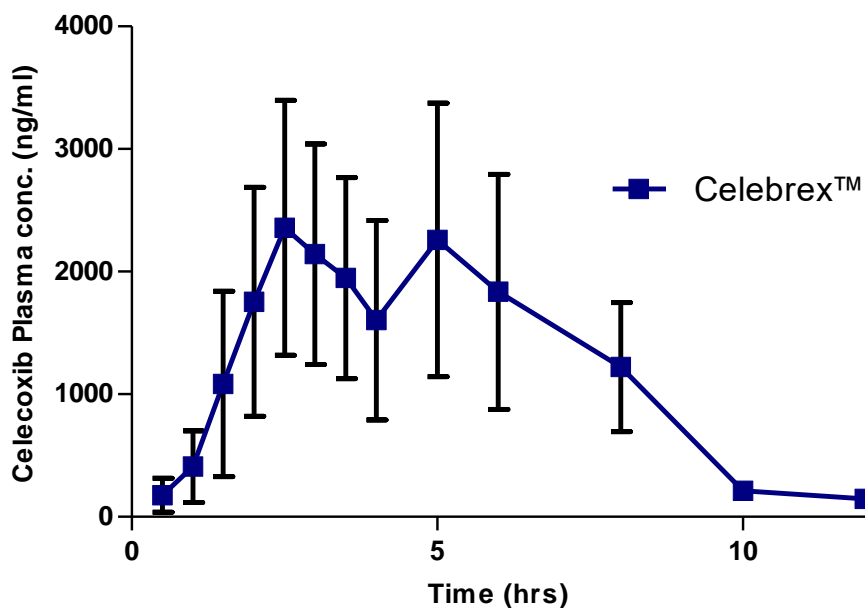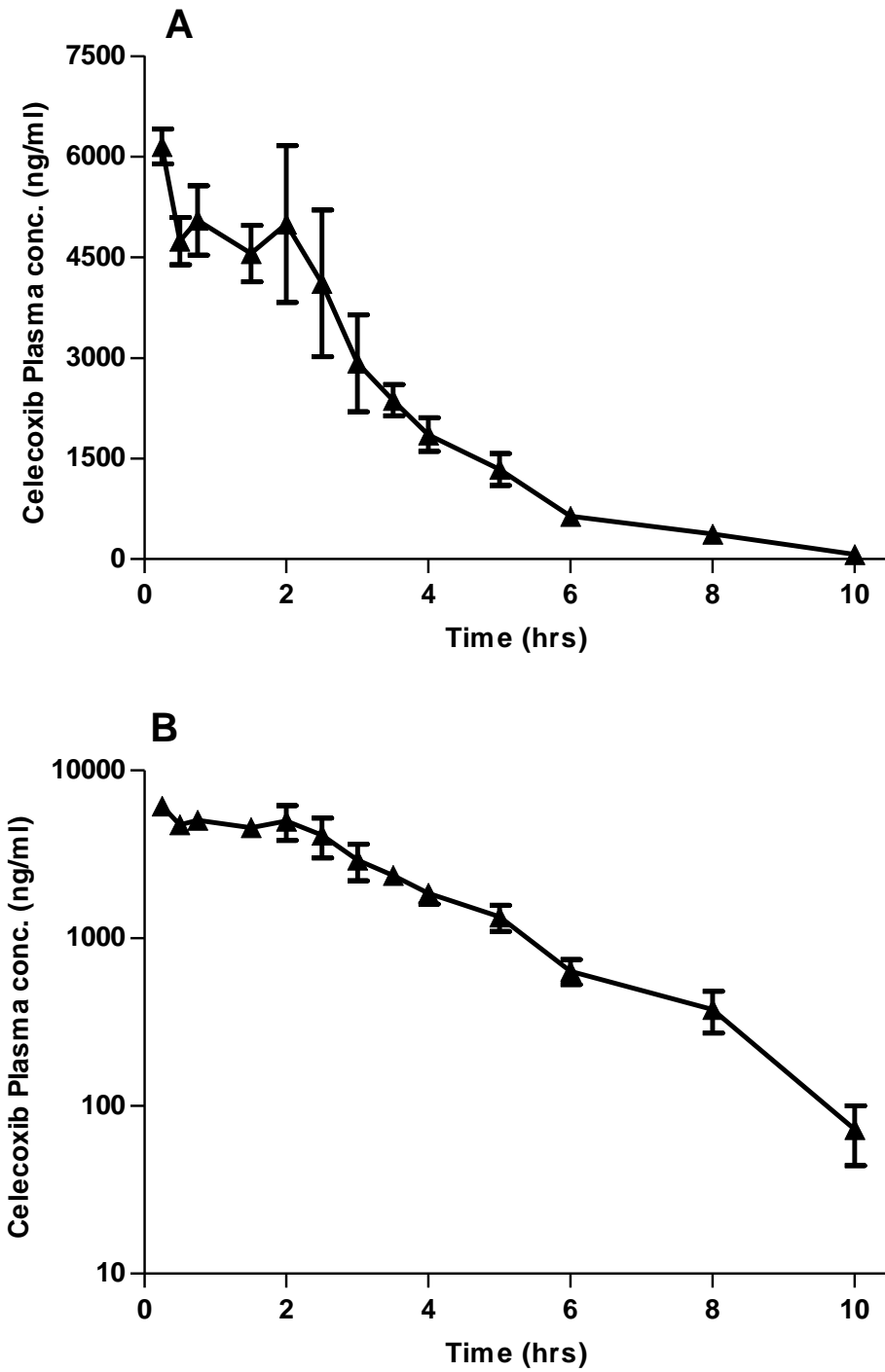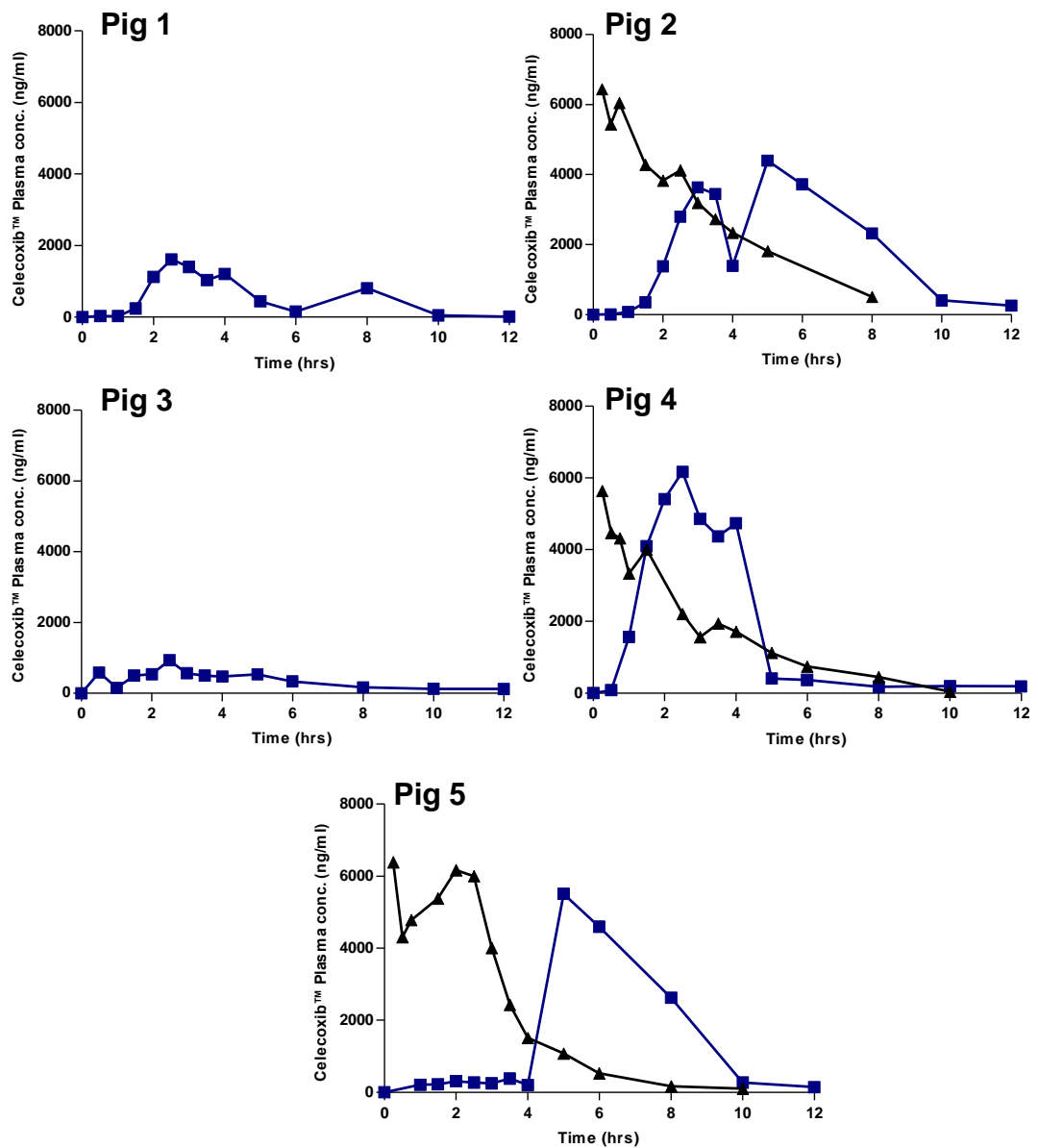*Figure 4-8 Individual celecoxib plasma concentration profile after oral administration of 100mg celecoxib as Celebrex™ Hard Capsules (blue squares) or as intravenous preparation (black triangles) to fasted pigs*

Figure 4-8 displays the individual plasma concentration-time profiles for each pig in the current study, as summarised in figures 4-6 and 4-7. From animals involved in the current study, oral bioavailability data for Celebrex™ capsules is available for five pigs, while intravenous data is only available for three of these animals. For pigs 1 and 3, the overall bioavailability of celecoxib from Celebrex™ appears to be substantially lower than that of the other pigs. Pig 4 also displays a significant lag time before appreciable levels of absorption are observed. Intravenous data appears to be more consistent for the three animals for which it is available. One point worth noting is the apparent secondary distribution phase observed with pig 5. This may be related to difficulties in cannulation of the ear vein observed in other animals and could potentially result from non-instantaneous perfusion from the site of administration. Observation of the individual profiles appears to confirm the observations of the mean profiles, with variable oral absorption but reduced variation in the intravenous dosing, indicating that the large variability may associated with pre-systemic, enteric processes.

Discussion

Celecoxib, as a BCS class II compound, is anticipated to display either solubility and/or dissolution rate limited bioavailability *in vivo*. Early *in vitro* screening in the current study supports this hypothesis, with low celecoxib solubility and incomplete dissolution of Celebrex™ in biorelevant media (figures 4-3 and 4-4). The solubility and dissolution observed in fed state media vs. fasted state media further support the prediction of solubility-limited bioavailability, as the presence of solubilising species in the fed state medium considerably enhances the solubility and dissolution. These data suggest that celecoxib has potential as a model compound in the assessment of the ability of various bioenabling formulations to improve *in vivo* absorption, and indeed celecoxib has widely been utilised for this purpose previously (Laine et al., 2016, Guzman et al., 2007, Nguyen et al., 2013, Subramanian et al., 2004, Rawat and Jain, 2004). As a BCS class II compound, it is also anticipated that celecoxib will demonstrate a significant positive food effect (Shono et al., 2009, Custodio et al., 2008, Benet, 2013). This hypothesis is supported by the enhanced solubilisation and dissolution observed for celecoxib *in vitro*. Early *in vitro* screening, therefore, suggests that celecoxib may be a useful model for characterisation of bioenabling formulations and in assessment of *in vivo* models of food effect bioavailability.

However, the extent of absorption of celecoxib in fasted state pigs seen here is higher and more variable than anticipated. A review of studies assessing celecoxib bioavailability in other species, namely dogs and humans, was conducted to provide further insights into and possible explanations for the current findings. The results of the studies assessed are summarised in table 4-4 and a detailed discussion of findings

of the current study, incorporating a comparison to these previously published results is provided in this discussion.

The bioavailability of celecoxib from Celebrex™ in the current study is higher than expected (89% absolute bioavailability for Celebrex™), relative to that anticipated based on *in vitro* screening, or, indeed, relative to the only comparable single dose studies of absolute bioavailability (25 – 40% in dogs), as described in table 4-4 (Paulson et al., 2001, Guzman et al., 2007). Near complete absorption suggests that bioavailability for the Celebrex™ formulation is not limited by solubility or dissolution rate in pigs. Bioenabling and dissolution enhancing formulations will, therefore, have limited efficacy in this case. Similarly, celecoxib does not appear to be a suitable candidate for assessment of food effect, as the large variability (CoV≈ 60%) will make it difficult to assess the moderate changes in bioavailability anticipated for a 'borderline' food-effect drug, like celecoxib. A possible explanation for the extensive absorption is that Celebrex™ itself may, in fact, be formulated to achieve maximal bioavailability. Excipients in the Celebrex™ product include PVP and SLS and most likely reflect a wet granulation production process and can improve wettability, while the relatively low particle size of 25µm means that dissolution may be optimised *in vivo* (Laine et al., 2016, FDA, 1998, Pfizer Inc., 2000). This relatively refined formulation already shows near optimal absorption in pigs, and further formulation attempts aimed at enhancing dissolution may merely display enhanced *in vitro* dissolution, with poor correlation to *in vivo* analysis.

A second factor to consider when analysing formulation performance is the high variation in the extent of bioavailability seen in this study. Celecoxib bioavailability

has previously been shown to be highly variable in multiple species. In fasted humans the coefficient of variation (CoV) in AUC has been shown to vary between 40-78%, while it reaches 40% in fasted dogs (table 4-4). High variability in oral absorption has also been observed in this study (CoV≈60% for Celebrex™), making it difficult to identify and quantify any true difference between formulations.

The causes of such large variations in bioavailability not entirely clear, but differences in metabolism mediated by enzymatic polymorphism have been shown to have a significant effect on celecoxib pharmacokinetics and exposure. While significant interspecies metabolic differences for celecoxib exist, genetic variation has been shown to play a role in bioavailability in multiple species. In humans, CYP2C9 is the primary enzyme involved in celecoxib metabolism (Paulson et al., 1999, Gong et al., 2012). Genetic variation in this enzyme reduces clearance and can more than double celecoxib exposure, with drug label warnings expressing caution in use of celecoxib in patients known to be poor 2C9 metabolisers owing to the risk of observing abnormally high plasma levels of celecoxib (Kirchheiner et al., 2003, Tang et al., 2001, Pfizer Inc., 2000). In such cases, using celecoxib at half the recommended lowest dose is advised.

In dogs, while different enzymes are responsible for clearance (CYP2D family), genetic polymorphisms still result in altered metabolism and up to 2 fold differences in exposure when dosed Celebrex™. While this goes some way toward explaining the variability in celecoxib exposure, even separation of dogs into different cohorts based on metabolism phenotype results in a CoV of 17-33% for animals dosed Celebrex™ (Paulson et al., 2001).

150

While, to our knowledge, the metabolism of celecoxib has not been assessed in the *in vivo* pig model or *ex vivo* in cultured porcine hepatocytes, such studies have been conducted with other Cyp2C9 substrates (Thörn et al., 2011). Of particular interest is the metabolism studies conducted with diclofenac, another NSAID which is principally metabolised by Cyp2C9 in humans (Brenner et al., 2003). *In vitro* metabolism of diclofenac was virtually non-existent in pig liver microsomes relative to human microsomes, while quantification of mRNA has demonstrated low expression of CYP2C42 in pig liver, suggesting that the CYP2C family has low hepatic activity in pigs and that extrapolation of *in vivo* pharmacokinetics for its substrates is difficult (Thörn et al., 2011, Bogaards et al., 2000). The principle enzymes involved in celecoxib metabolism in pigs are unknown though it is possible that they may contribute to the high variability which makes detection of formulation absorption effects challenging. This theory is supported by comparing the variability in plasma concentrations observed in the oral, relative to the intravenous, leg of the current study. High variability (CoV≈60%) is observed in Celebrex™ oral bioavailability, while the variability in exposure is considerably lower in the i.v. study leg (CoV≈16%). This indicates that such large variability is principally associated with pre-systemic events, which may include absorption associated events such as dissolution and permeation, along with enteric and hepatic first pass metabolism.

One potential limitation of this assessment is, however, the absence of intravenous data for pigs 1 and 3, which are the principle source of overall variability in the oral pharmacokinetics (figure 4-8). The individual profiles are presented here to highlight potential sources of the considerable variability in overall oral bioavailability. The

high variability observed in the oral plasma concentrations in the current study appears primarily attributable to these two individual pigs (pigs 1 and 3), which appear to display significantly lower overall bioavailability compared to other animals. These pigs received the Celebrex™ capsules in different study legs (pig 1 received Celebrex™ in week 1, while pig 3 received Celebrex™ in week 2) and no anomalies were noted during dosing, suggesting administration is unlikely to be the primary source of variability in this case. It is worth noting that for both these pigs, difficulties in cannulation of the ear vein for i.v. administration, though how this would relate to overall bioavailability is unclear. The absence of i.v. data for pigs 1 and 3 due to this inability to cannulate ear veins means limit our ability to conclusively relate these variable plasma concentrations to pre-systemic/ enteric effects, as there is no comparative i.v. data in animals which appear to display substantially lower plasma concentrations.

While the extensive bioavailability and high variability contribute to limiting the suitability of celecoxib, and the Celebrex™ formulation in particular, as a model compound for the assessment of bioenabling formulations in pigs, discussion of whether this study accurately predicts the likely performance in humans is of merit. Inter-species variation in bioavailability, such as that seen here between pigs and dogs, is common. It is not always possible to determine which species most accurately reflects *in vivo* performance in humans. The dog model, however, has significant limitations in predicting celecoxib bioavailability in humans. In particular the shorter intestinal transit time (approximately half that of humans) may not provide adequate time for poorly soluble drugs to completely dissolve, which may

limit bioavailability (Sjogren et al., 2014). Dissolution plays a key role in determining the extent of celecoxib absorption in dogs, with significant increases in bioavailability when a solution is dosed and large fed state increases in bioavailability (Paulson et al., 2001). No similar significant food effect is seen in humans, suggesting extensive absorption already occurs in the fasted state (FDA, 1998, Shono et al., 2009). This indicates that solubility and dissolution may not play as critical a role in determining celecoxib exposure in humans as it does in dogs. The high levels of bioavailability in the fasted pig model may, therefore, more accurately reflect celecoxib bioavailability in humans. However, with no measure of absolute bioavailability in humans available, this cannot be stated with complete certainty. The results from the studies used in this discussion are summarised in table 4-4 below.

*Table 4-4 Summary of published celecoxib food effect pharmacokinetic and bioavailability data (*concentrations originally reported as mcg/ml; converted to ng/ml for comparison) (EM; dogs phenotyped for extensive metabolism, PM; dogs phenotyped for poor metabolism, HFF; high fat fed, MFF; medium fat fed, LFF; low fat fed)*

Subjects	Formulation	Dose	Food State	n	C_{max} (ng/ml)		T_{max} (hours)		AUC (ng.h/ml)		Bioavailability (%)		Comment	Ref.
					Mean	CoV	Mean	CoV	Mean	CoV	Mean	CoV		
Human	Celebrex™	100mg	Fasted	24	455	0.60	2.6	0.46	5127	0.78	-	-	High CoV, no significant food effect.	(FDA, 1998)
			Fed	24	747	0.51	5	0.48	5419	0.71	-	-		
Beagle dog (EM)	Solution	5mg/kg	Fasted	3	820	0.46	0.67	0.43	2630	0.38	63.7	0.27	Significant differences in exposure depending on metabolism phenotype. Increase in bioavailability from solution compared to solid dosage forms. Higher variability with solid dosage forms. Neat chemical comparable to capsules in dogs.	(Paulson et al., 2001)*
	Neat chemical			6	230	0.53	1.5	0.32	950	0.85	21.7	0.60		
	Capsule			6	280	0.17	1.3	0.18	970	0.25	24	0.17		
Beagle Dog (PM)	Solution	5mg/kg	Fasted	3	1320	0.03	0.5	0	10500	0.26	88.2	0.11		
	Neat chemical			6	580	0.63	3.3	1.26	4400	0.44	39.4	0.43		
	Capsule			6	320	0.30	1.3	2.07	3000	0.24	27.2	0.33		

Species	Formulation	Dose	Food	N	Cmax	CoV	Tmax	CoV	AUC	CoV	F	CoV	Comments	Reference
Beagle dog (EM)	Neat Chemical	5mg/kg	Fasted	3	230	0.30	1.5	0.34	1700	0.71	27.4	0.92	Significant food effects seen in dogs regardless of metabolism status. Bioavailability increases with increasing fat content and hence solubility. Difference in exposure seen with different metabolism phenotype.	(Paulson et al., 2001)*
			LFF	3	670	0.59	3	0.28	4100	0.63	59.9	0.46		
			MFF	3	580	0.41	5.3	0.22	4100	0.50	60.4	0.33		
			HFF	3	660	0.28	4	0.51	5000	0.48	74.4	0.28		
Beagle Dog (PM)	Neat Chemical	5mg/kg	Fasted	3	490	0.35	7.5	1.22	3900	0.17	42.2	0.20		
			LFF	3	890	0.09	3.8	0.50	8100	0.14	87.5	0.18		
			MFF	3	760	0.11	4.7	0.25	6300	0.08	67.9	0.06		
			HFF	3	890	0.05	7.3	0.16	8800	0.09	93.7	0.02		
Human	Celebrex™	200mg	Fasted	24	806	0.50	2.44	0.34	5994	0.39	-	-	High CoV, no significant food effect even with high fat meal and higher dose	(Paulson et al., 2001)
			HFF	24	1042	0.34	3.42	0.37	7318	0.38	-	-		
Dog	Celebrex™	5mg/kg	Fasted	6	654	0.30	1.25	0.70	7663	0.41	40.1	0.38	Absolute bioavailability of 40%	(Guzman et al., 2007)
Pig	Celebrex™	100mg	Fasted	5	3726	0.62	3.5	0.39	16539	0.62	89.23	0.62	Absolute bioavailability of 89.23% Large coefficient of variation	This study

Conclusion

Biorelevant *in vitro* screening of celecoxib solubility and dissolution suggests that this BCS class II compound will display solubility limited oral absorption, poor bioavailability in the fasted state and a positive food-effect. This early screening initially suggests that celecoxib, would indeed be a good marker compound for assessment of bioenabling formulations and food-effect bioavailability. Oral dosing of the commercial Celebrex™ formulation in the fasted pig, however, displayed high, but variable oral bioavailability. The overall bioavailability of celecoxib in previous animal and human studies appears to be highly variable, while previous dog studies have not accurately predicted celecoxib pharmacokinetics in humans in terms of food effects. The pig model, while also variable, may more accurately reflect bioavailability of celecoxib in humans, though confirmatory studies to this end would be required. Overall, the results of the current study suggest that celecoxib would not be an appropriate model compound to screen the performance of bioenabling formulations or in the assessment of food-effect in the pig model.

Chapter 5 : The utility of a porcine model for predicting food dependent bioavailability: Case study with fenofibrate

Introduction

The concomitant administration of oral dosage forms with food can have a significant impact on drug pharmacokinetics and bioavailability relative to the fasted state. With oral drug delivery continuing to be the method of choice for drug administration, understanding the effects food has on the biopharmaceutical aspects of drug delivery is key to the drug development process as well as the effective and rational use of medicines in the clinical setting (Fleisher et al., 1999, Abuhelwa et al., 2017).

The intake of food has many and varied effects on drug absorption depending on drug and formulation characteristics, as well as the impact of physiological and physicochemical changes in the post-prandial GIT (Fleisher et al., 1999, Custodio et al., 2008, Gu et al., 2007, Abuhelwa et al., 2017, Lentz, 2008, Varum et al., 2013). This can either result from direct interaction of food components with the dosage form, or indirectly, with effects mediated by food-induced changes in gastrointestinal physiology or the physicochemical composition of gastrointestinal fluid. These physiological effects can include;

1) Slower gastric emptying resulting in extended T_{max} and a reduced C_{max}

2) Increase in gastrointestinal fluid volume, increasing the volume available for solubilization/dissolution

3) Increased gastric pH, altering the solubility of ionisable compounds

4) Increased secretion of biliary lipids (bile, cholesterol, phospholipids etc.) resulting in increased solubilisation for poorly soluble drugs

5) Increased splanchnic blood flow, which can result in changes to drug metabolism

158

These effects can cause significant changes in drug absorption and bioavailability depending on the prandial state in which a drug is taken.

The understanding of the effects food has on pharmacokinetics is consequently a critical factor in assessing the clinical potential of new medicines and designing a food effect resistant formulation early in drug development can both provide a commercial advantage and prevent costly reformulation later in the product lifecycle. Numerous challenges exist in the development of compounds which demonstrate a food effect, such as the potential for sub-therapeutic levels where a high-fat meal is required to increase absorption, unwanted side-effects where the bioavailability of a compound with a narrow therapeutic index is enhanced, or where a competitor holds a commercial advantage due to the absence of restrictions with regard to dosing with food (Lentz, 2008). Predicting the likelihood of a food effect and performing food effect bioavailability studies in early drug development is essential to provide a better understanding of the absorption process for a specific drug candidate, including what factors are critical to its absorption and anticipating the expected pharmacokinetic variability. Getting an early read on the anticipated food effect will also provide an opportunity to formulate away this effect and/or ensure that drug product labelling contains appropriate recommendations with regard to dose administration with food in the post-licensing environment (Mathias et al., 2015).

Accordingly, the FDA have provided guidelines on how to design clinical trials to investigate food effects, recommending dosing in both fasted and fed states. The FDA guidance defines that a food-effect is established if the 90% confidence intervals for

the ratio of population geometric means, based on log-transformed data, for either

$AUC_{0\to\infty}$ or C_{max} fall outside the 80-125% bioequivalence limits relative to the

reference, i.e. the same formulation administered in the fasted stated (FDA, 2002).

The fed state represents dosing post ingestion of a high fat, FDA standard breakfast,

containing 800 – 1000 kcal with approximately 50% of total calories coming from fat,

to maximise potential for demonstrating a food effect (FDA, 2002). While this

regulatory guidance is essential when assessing the impact of food on absorption in

the clinical setting, prediction of food effects is also important in drug development,

where information is needed before initiation of the clinical program.

The Biopharmaceutical Classification System (BCS) and Biopharmaceutical Drug

Disposition Classification system (BDDCS) provide useful predictions of potential food

effects based on drug physicochemical properties, as summarised in figure 5-1 (Wu

and Benet, 2005, Amidon et al., 1995). The anticipated effects are predicted by the

most likely limiting factor for bioavailability, namely solubility or dissolution for

BCS/BDDCS class II compounds, permeability for class III compounds, or a

combination thereof for BCS class IV compounds. An overall delay in T_{max} and reduced

C_{max} for highly bioavailable compounds can be associated with a delayed gastric

emptying (Custodio et al., 2008). While this tool does not capture all the potential

effects of food and does not take formulation factors into account, it is the most

widely utilised simple tool to predict food effect behaviour, and is estimated to be

accurate in approximately 70% of cases (Benet, 2013).

Figure 5-1 Predicted effect of high fat meal on oral pharmacokinetics by BCS/BDDCS Class; adapted from Custodio et al. (2008)

In order to provide a more accurate prediction of food effects a range of techniques have been developed including traditional *in vitro* dissolution apparatus in conjunction with biorelevant media which mimic the post-prandial environment of the gastrointestinal tract (Kleberg et al., 2010, Nicolaides et al., 1999, Kalantzi et al., 2006, Mathias et al., 2015, Baxevanis et al., 2016, Sunesen et al., 2005, Markopoulos et al., 2015). More advanced apparatus, such as dynamic gastrointestinal models have also been utilised to good effect, though the complexity and cost of these methods hinder their widespread utility (Brouwers et al., 2011, Lyng et al., 2016, Kostewicz et al., 2014b). The limitations of *in vitro* techniques in accurately reflecting the complexity of *in vivo* gastrointestinal environment physiology has led to the integration of these *in vitro* techniques with advanced *in silico* physiologically based pharmacokinetic (PBPK) models. These models vary significantly in design and in

complexity, but the overall aim is to combine molecule physicochemical and biopharmaceutical descriptors with data garnered from *in vitro* analysis, as well as *in vivo* factors such as gastrointestinal transit and luminal conditions, through the use of differential equations. These models have been widely utilised in recent years to analyse and predict *in vivo* food effects with varying degrees of success (Shono et al., 2009, Otsuka et al., 2013, Jones et al., 2006, Fei et al., 2013, Pandey et al., 2014, Kostewicz et al., 2014a, Patel et al., 2014, Wagner et al., 2012, Shono et al., 2010).

Despite the recent proliferation of *in vitro* and *in silico* techniques, the most commonly utilised pre-clinical approach to accurately anticipate food effect is the utilisation of *in vivo* bioavailability studies. To date, the most widely utilised animal model of food effect is the dog, despite the significant dietary and gastrointestinal anatomical and physiological differences relative to humans (Kararli, 1995, Lui et al., 1986, Dressman, 1986, Sjogren et al., 2014). Lentz *et al.* (2007) have developed a protocol for conducting food effect studies in dogs comparing the C_{max} and AUC in the fed and fasted state in dogs to that observed in humans. A range of nine compounds which displayed positive, negative and no food effects were investigated in this study and an optimal protocol involving pre-treating with pentagastrin and feeding of 50g of a homogenised FDA high fat, high caloric meal was identified. While this model was useful in identifying both positive and negative food effects, it was less useful in correctly identifying compounds which do not display any food effect (Lentz et al., 2007). This is an indication that dogs may be more sensitive to food effects than humans, a finding also identified elsewhere (Mathias et al., 2015).

In recent years, the pig has become increasingly popular in pre-clinical bioavailability studies owing to the perceived similarities in gastrointestinal anatomy and physiology between pigs and humans (Walters et al., 2011, Puccinelli et al., 2011, Suenderhauf and Parrott, 2013, Sjogren et al., 2014). Christiansen *et al.* (2015) have recently investigated the utility of the minipig as a model of food effect, using an approach similar to that of Lentz *et al.* (2007), utilising a homogenised FDA meal, as well as using a nutritional drink supplement, with both treatments applied both with and without pentagastrin. While their findings suggest that the use of pentagastrin is unnecessary in minipigs, and has an insignificant effect on absorption, overall the research demonstrates that the dog protocol cannot simply be transferred to minipigs, but requires further refinement (Christiansen et al., 2015). One of the challenges of the utility of the pig model is the apparent variability in gastric emptying which have been observedto , with values from as low as 1-2 hours to as high as 24 hours reported in the literature for disintegrating tablets (Davis et al., 2001, Oberle and Das, 1994). Both Christiansen *et al.* (2015) and Henze *et al.* (2018a) co-administered paracetamol (acetaminophen) with food, as a marker of gastric emptying rate, as it is rapidly absorbed upon entering the small intestine, but observed no difference in the fed and fasted groups, leading to the suggestion that the caloric content of the utilised meal was not high enough to delay gastric emptying. In light of the current research into the utility of the pig as a model of bioavailability, and specifically with regard to food effect, this study proposes to assess the utility of the landrace pig in such a scenario.

The aims of the current study were, firstly to assess the ability of a of the landrace pig in evaluating a proven positive food effect in humans using fenofibrate as a lipophilic marker BCS class II compound, and the FDA breakfast as a food source. The FDA breakfast is a common food source in such studies, consisting of high fat, high caloric meal designed to maximise the extent of food effect, particularly for poorly water soluble compounds. Secondly, standard pig feed was also investigated as an alternative food source, based on previous preliminary studies where significant quantities of residual food were observed post-mortem in 'fasted' pigs. A fed state incorporating pig pellet feed was, therefore, investigated in order to assess the potential impact of this residual food material. Thirdly, the effect of food on the pharmacokinetics of paracetamol is assessed, both as a marker of the gastric emptying rate in the fasted and fed states and as a comparator BCS class I compound. Significant increases are expected in both C_{max} and bioavailability for the BCS class II compound fenofibrate in the fed state, while food is not expected to have any impact on the extent of paracetamol bioavailability as an immediate release BCS class I compound. Feeding is, however, anticipated to reduce the rate of absorption of the immediate release BCS class I compound, due to delayed gastric emptying, meaning paracetamol also acts as a useful indicator of gastric emptying. Finally, the effect of three different fasting regimens, as well as one fed state condition, on the gastrointestinal contents of pigs were evaluated in order to determine the ability of each regimen to ensure a complete fasted state and to elucidate the differences in gastrointestinal fluid characteristics in both the fasted and fed state.

Materials and methods

Materials

Paracetamol, 2-acetamidophenol and fenofibric acid were purchased from Sigma-Aldrich (Ireland) Ltd. Fenofibrate was purchased from Kemprotec Ltd. (UK). Hard gelatin capsules (Size 0) were obtained from Capsugel (Coni-Snap®). Lipantil™ Micro 67mg hard capsules and Paralief™ 500mg tablets were commercially sourced from local pharmacies. All food components used in preparing FDA recommended breakfast were purchased commercially. All other chemicals and solvents were of analytical grade or HPLC grade respectively and were purchased from Sigma–Aldrich (Ireland).

Oral bioavailability in pigs

The study was carried out under licences issued by the Health Products Regulatory Authority (HPRA), Ireland, as directed by the Cruelty to Animals Act, Ireland and EU Statutory Instruments (Licence number AE19130/P058). Local University ethical committee approval was also obtained. The study was a non-randomised, one sequence, three-way crossover design, where the pigs were dosed in the fasted state on week one, fed half a standard high-caloric, high-fat FDA breakfast (table 5-1) in week two and fed 175g pig weanling pellet feed in week three. The mass of FDA breakfast fed equated to approximately 18 – 20g/kg of body weight and was not adjusted for body weight.

165

Component	Approximate weight (g)	Approximate Total Calories (kcal)	Approximate Calories from fat (kcal)
One slice of bacon	30	70	53.9
1 slice buttered toast	45	100	27
1 fried egg	60	92	64.4
4oz (118ml) whole milk	122.5	70	33.6
2oz hashed brown potatoes	57.5	112	45
Total	**315g**	**444 kcal**	**223.9 kcal**

Six male landrace pigs (15.7–17.3kg, mean 16.53kg) were sourced locally and housed individually at the University's Biological Services Unit. Pigs were fed approximately 175g of standard weanling pig pellet feed twice daily. The final feed was given 24 hours prior to dosing. As part of the study design any remaining food was to be removed 16 hours before dosing, however no food remained at this point in any of the study legs.

On day 1, an indwelling intravenous catheter was inserted into the jugular vein, under general anaesthesia as previously described (Faisal et al., 2013). Following an overnight fast on day 3, oral formulations of Lipantil® Micro 67mg hard gelatin capsules and Paralief™ 500mg tablets with the aid of a dosing gun, after which the pigs received 50mL of water *via* syringe. After dosing, pigs were returned to their pens. Blood samples (4mL) were collected at time zero (pre-dosing) and 0.5, 1, 1.5,

2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12 and 24 hr post-dosing. Water was available *ad libitum* throughout the study period and the animals were fed with 175g of pig weanling pellet feed 8 hours post-dose. All blood samples were collected in heparinised tubes (Sarstedt, Germany) and immediately centrifuged at 3220*g* for 5 min at 4°C (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany). Plasma was collected and stored at −80°C prior to analysis.

A six-day washout period was observed between each leg of the study. All animals remained in good health throughout the study. However, problems arose with sampling *via* the indwelling cannula during the first week for three of the pigs, for one additional pig during week two of the trial and for one further additional pig during the third week. This means that three pigs completed the fasted leg of the study, two of which also completed the FDA meal leg, with a full crossover including the leg involving pig food only achieved in one individual pig.

Pig fluid collection

Upon completion of the pharmacokinetic study all animals were euthanized by intravenous injection of pentobarbital sodium followed by potassium chloride. The peritoneal cavity was exposed by midline incision and the stomach and small intestine were located and isolated. Occluding ligatures were applied proximal to the cardiac sphincter and distal to the pyloric sphincter and at the proximal and distal ends of the small intestine. Once both ends were secured, both the stomach and small intestine were removed from the peritoneal cavity. The luminal fluid was collected in sterile 50ml sample tubes and frozen. Thawed samples were subsequently homogenised using a T25 Ultra-Turrax® homogeniser (IKA®-Werke

GmbH & Co. KG, Germany) probe for 5 minutes at 200rpm. Samples were centrifuged at 3220g for 10 min at ambient temperature (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany) to separate solid content from liquid content. Liquid supernatant was macroscopically observed to be free from solids and was removed and placed in 50ml sterile tubes. Solid content was measured using lab balances, while volume of liquid content was measured in a graduated cylinder. Fluid pH was measured using a calibrated Jenway 3510 pH meter.

Differing dietary states were induced prior to euthanasia to assess the impact of varying fasting regimens on the remaining food in the gastrointestinal tract at time of dosing. This procedure was also carried out with pigs from previous studies conducted in this thesis (chapters two and four). The following feeding regimens were evaluated;

A. 24 hour fast, once daily feeding (24 hour o.d.); Pigs fed approximately 500g of pellet feed once daily, with final feeding 24 hours prior to euthanasia (n=5; from study carried out in chapter four of this thesis)

B. Fed state, twice daily feeding (4 hour b.d.); Pigs fed approximately 175g of pellet feed twice daily, final feeding consisted of 500g of pellet feed approximately 4 hours prior to euthanasia (n=3; current study)

C. 16 hour fast, twice daily feeding (16 hour b.d.); Pigs fed approximately 175g of pellet feed twice daily, with final feeding 16 hours prior to euthanasia (n=3; current study)

D. 24 hour fast, twice daily feeding (24 hour b.d.); Pigs fed approximately 175g of pellet feed twice daily, with final feeding 24 hours prior to dosing (n=6; from study carried out in chapter two of this thesis)

Quantitative analysis of fenofibrate

The pharmacokinetic evaluation of fenofibrate was based on the quantification of fenofibric acid, the major active metabolite of fenofibrate, using a validated HPLC-UV method, as previously described (Griffin et al., 2014). Briefly, 0.5ml plasma was spiked with 20μl of a sulindac 100μg/ml solution in methanol as an internal standard. Proteins were precipitated through addition of 0.5ml of 25% NaCl solution and 1ml of 1% H_3PO_4 in methanol with thorough mixing. Samples were centrifuged at 11,500g for 9 min (Hermle z233 M-2 fixed angle rotor centrifuge; HERMLE Labortechnik GmbH, Wehingen, Germany). The clear supernatants were injected onto a Synergi Fusion C18 reversed phase column (250 × 4.6mm, 4μm) (Phenomenex Inc., Macclesfield, UK) using an Agilent 1200 series HPLC system (Agilent Technologies, Santa Clara, CA, USA). The mobile phase consisted of 80% methanol: 20% water (adjusted to pH 2.5 with phosphoric acid) at a flow rate of 1ml/min, resulting in elution of fenofibric acid and fenofibrate at 6.5 and 10.5 min, respectively. UV detection was performed at 286nm. The analysis showed linearity over the range of 50–2000ng/ml with an LOQ of 80ng/ml and extraction recoveries were ≥95%.

Quantitative analysis of paracetamol

The plasma concentrations of paracetamol were determined by a reversed-phase HPLC method. The HPLC system comprised an Agilent 1200 series compact HPLC equipped with a variable wavelength detector. A Synergi, C18 reversed phase column (250 × 4.6mm, 4μm) (Phenomenex Inc., Macclesfield, UK) column was used for the chromatographic separation. Mobile phase consisted of 0.1% w/v acetic acid: methanol (70:30 v/v) and was used at a flow rate of 1ml/min. The column

temperature was not controlled and the detection wavelength was set at 254nm. The retention time for paracetamol was approximately 6.5 minutes and for internal standard was 11 minutes. Due to the strong retention of fenofibric acid with this buffer/ column combination, a wash run was performed after every three sample runs. This was a 20 minute cleaning run with a gradient from 70% acetic acid and 30% methanol to 20% acetic acid and 80% methanol over five minutes. The system was held at this composition for five minutes before returning to the original gradient over five minutes and re-equilibrating the column at this gradient for five minutes.

Paracetamol was extracted from the plasma samples by liquid-liquid extraction. 100µl of plasma sample was transferred to a 1.5ml Eppendorf microcentrifuge tube. 60mg sodium chloride, 10 µl internal standard (250 µg/ml, 2-acetamidophenol) were added to the tube. Sodium chloride was added to precipitate and denature protein before extraction. The tubes were mixed using a benchtop vortex. 1000µL of ethyl acetate was added to extract paracetamol. The tubes were shaken for 30 seconds using a vortex mixer followed by centrifugation at 11,500g for 9 min (Hermle z233M-2 fixed angle rotor centrifuge, HERMLE Labortechnik GmbH, Wehingen, Germany) at ambient temperature. The supernatant was transferred to a round-bottom polypropylene vial and evaporated to dryness under a stream of nitrogen at 60°C. A second extraction step was carried out by adding 1000µL ethyl acetate to the precipitate containing tube, vortexing again for 30 seconds and centrifuging under similar conditions. The supernatant was transferred to the corresponding round-bottom polypropylene vial from the previous step and evaporated to dryness under a stream of nitrogen at 60°C. The residue was dissolved in 100µl mobile phase and

20μl was injected into the column. The limit of quantification by this procedure was 500ng/ml and the assay was linear between 500ng/ml and 15,000ng/ml. The extraction efficiency was greater than 89% across the concentration range.

Pharmacokinetic data analysis

The total exposure after oral administration was estimated by calculating AUC for fenofibric acid and for paracetamol using Prism (ver. 5, GraphPad Software Inc., La Jolla, Ca.). The peak plasma concentrations (C_{max}) and the time for their occurrence (T_{max}) were noted directly from the individual plasma concentration vs. time profiles. The Absolute Bioavailability (F_a) of fenofibrate was calculated according to equation 5-1 below, using previously published intravenous data (O'Shea et al., 2015):

$$Fa = \left(\frac{AUC_{oral}}{AUC_{i.v.}} \right) \cdot \left(\frac{Dose_{i.v.}}{Dose_{oral}} \right) \qquad (5\text{-}1)$$

All pharmacokinetic parameters are reported as mean ± SD, where possible, with the exception of T_{max}, which is reported as median (range). Where the limited number of animals prohibited accurate estimation of the error associated with the mean due to loss of animals to the study, the pharmacokinetic parameters are presented as mean (range).

Food effect was calculated using the fold difference (FD) in the AUC in fed vs the fasted state using equation 5-2:

$$FD = \left(\frac{AUC_{fed}}{AUC_{fasted}} \right) \qquad (5\text{-}2)$$

Fold differences (FD) are presented, where possible, as mean FD ± standard error of the fold difference (SE_{FD}) as calculated by equation 5-3:

171

$$SE_{FD} = FD \times \sqrt{\frac{SE_{fed}^2}{AUC_{fed}^2} + \frac{SE_{fasted}^2}{AUC_{fasted}^2}} \qquad\qquad (5\text{-}3)$$

Where FD is the mean fold difference in food effect, AUC_{fed} and AUC_{fasted} are the

represent the mean AUC in the fed and fasted states and SE_{fed} and SE_{fasted} represent

the standard errors corresponding to these values. In cases where insufficient sample

size did not allow for calculation of a standard error, the FD is presented solely as the

mean FD with no associated error.

Results

Effect of food on oral pharmacokinetics of fenofibrate in pigs

Figure 5-2 represents the plasma concentration profiles obtained following oral administration of 67mg fenofibrate as Lipantil® Micro to fasted pigs (n=3, mean ± range), pigs fed with half of a high-fat, high-caloric FDA standard breakfast, as described in table 5-1, 30 minutes prior to dosing (n=2, mean ± range) and pigs fed standard pig food (175g) 30 minutes prior to dosing (n=1). Absorption of fenofibrate in the fasted state begins rapidly, with detectable plasma concentrations of fenofibric acid observed as early as 30 minutes, relative to pigs fed the FDA meal, which displays an initial lag of 2.5 hours before quantifiable concentrations are observed. Dosing following pig food appears to significantly delay fenofibrate absorption. Drug levels in plasma were below the limit of quantification for 8 hours post dose, and the maximal observed concentration occurs at 24 hours. Given that highest observed concentration occurs at the final sampling time-point, accurate determination of AUC, C_{max} or T_{max} was not feasible in this case.
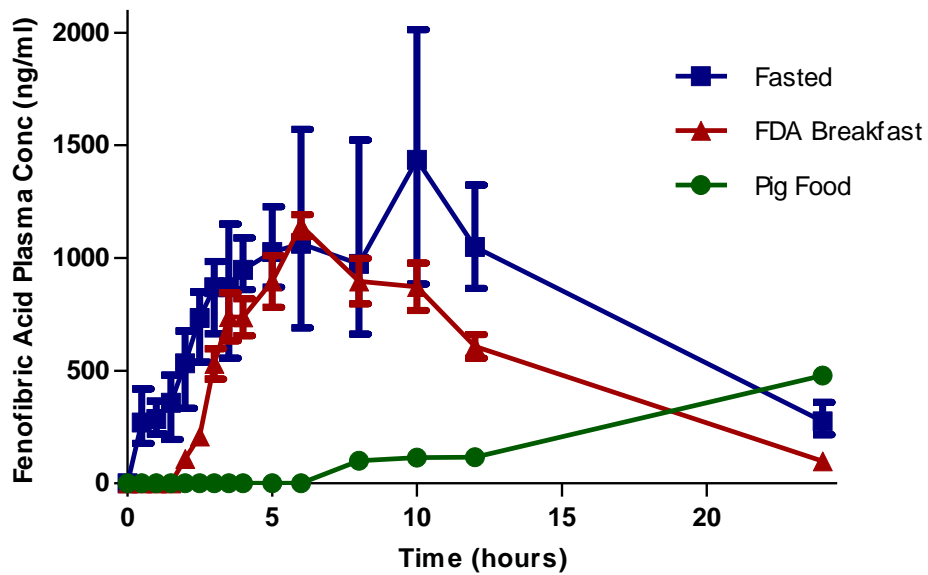
Figure 5-2 Plasma concentration of fenofibric acid vs. time profiles after oral administration of 67 mg fenofibrate as Lipantil® Micro, (■) indicates fasted state (n=3, mean ± range), (▲) indicates fed half FDA standard breakfast (n=2, mean ± range), (●) indicates fed 175g weanling pellet feed (n=1).

The key pharmacokinetic parameters for fenofibric acid are summarised in figure 5-3 and table 5-2. Absolute bioavailability has been calculated from i.v. data previously published from a similar study (Griffin et al., 2014, O'Shea et al., 2015). The data from the standard pig feed leg of the study has been omitted from this summary due to the difficulty in calculating accurate pharmacokinetic data when the final sampling time-point corresponds to the observed T_{max}. For the animals dosed 30 minutes following intake of a high fat breakfast there appears to be slight reductions in both C_{max} and overall bioavailability. The mean observed C_{max} in the fasted state of 1489.29ng/ml reduced to 1143.72ng/ml in animals fed the FDA breakfast, while the overall bioavailability reduced from an average of 38.81% to 25.08% (table 5-2). Both these values are substantially lower than that reported in a similar study carried out with Lipantil® Micro in the fasted state, where a mean (±SD) C_{max} of 2691.35 ±

174

728.01ng/ml and overall bioavailability of 66.1 ± 3.5% were observed (chapter three of this thesis). The T_{max}, however, in both studies seem to correspond well, with prolonged absorption observed in the fasted state, and with two of the three animals demonstrating a T_{max} which occurs after feeding at 8 hours in the current study. Partial AUC analysis, up to 8 hours post dose was also carried out in order to analyse if there was any difference in the rate of absorption, similar to the partial AUC analysis carried out in chapter three. A similar trend was noted in comparing fasted to fed data at both 8 hours and 24 hours, where a slight reduction in mean AUC in the fed state (4857 ng.h/ml) was observed relative to the fasted state (6268 ng.h/ml), a similar ratio to that of overall bioavailability. Thus, while an initial lag is observed in the fed state, it does not appear to have had a significant impact on either the rate or extent of bioavailability, relative to the fasted state.

The limited number of animals remaining at the end of this study prohibits the use of statistical tests of significance, however trends in both animals which have completed the current crossover follow that of the mean data. This observation runs contrary to the expected result, where fenofibrate bioavailability would be expected to increase in the fed state. This does, however, correspond to the lack of a consistently observed significant food effect for either atazanavir (expected positive food effect) or pravastatin (expected negative food effect) in a comparable food effect study carried out in minipigs (Christiansen et al., 2015).
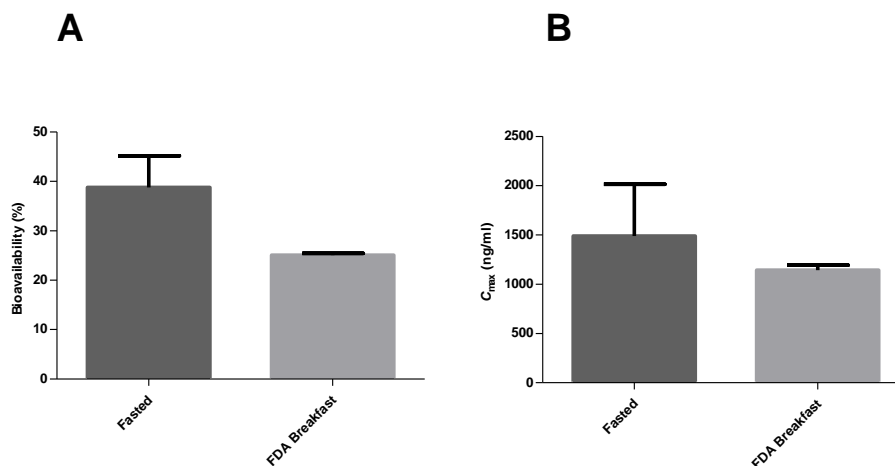
Figure 5-3 Bioavailability at 24 hours (A) and C_{max} (B) of fenofibrate from Lipantil® Micro 67mg in fasted (n=3, mean ± range) and FDA breakfast fed pigs (n=2, mean ± range)

Table 5-2 Summary of pharmacokinetic parameters after intravenous administration of 25mg of fenofibrate to fasted pigs (mean ± SD, n = 4) and after oral administration of 67mg of fenofibrate as Lipantil® Micro to fasted pigs (n=3, mean (range)) and to pigs fed FDA breakfast (n = 2, mean (range))

[a] *Intravenous data reproduced from O'Shea et al. (2015)*

[b] *Median (range)*

[c] *No range – both T_{max} occurred at 6 hours*

Intravenous pharmacokinetic Parameters[a]		Oral pharmacokinetic parameters		
V_c (L/kg)	0.345 ± 0.02504		**Fasted**	**FDA Breakfast**
K_{el} (hr^{-1})	0.221 ± 0.064428	C_{max} (ng/mL)	1489.29 (884.44 – 2012.53)	1143.72 (1094.42 – 1193.03)
K_{el} (hr^{-1})	0.099 ± 0.038687	T_{max} (hours)[b]	10 (6 – 10)	6[c]
K_{el} (hr^{-1})	0.35125 ± 0.241289	Bioavailability (%)	38.81 (29.16 – 45.20)	25.08 (24.72 – 25.44)
$AUC_{0\rightarrow24hrs}$ (ng.h/ml)	18382 ± 4591	$AUC_{0\rightarrow24hrs}$ (ng.h/ml)	19109 (14356 – 22254)	12350 (12173 – 12527)
		$AUC_{0\rightarrow8hrs}$ (ng.h/ml)	6268 (4572 – 8162)	4857 (4416 – 5297)

Effect of food on oral pharmacokinetics of paracetamol in pigs

In the current study, paracetamol was co-administered with fenofibrate in order to both act as a control compound, which is not expected to display a significant change in overall bioavailability when taken with food and to provide an estimate of gastric emptying and the impact of food on this dynamic physiological process. Paracetamol is rapidly absorbed on entry into the small intestine and has widely been used as a marker of gastric emptying, with T_{max} corresponding to approximately 50% gastric emptying (Christiansen et al., 2015, Medhus et al., 2001). Figure 5-4 represents the plasma concentration-time profile for paracetamol in pigs dosed in the fasted state, as well as pigs fed with either the FDA breakfast or standard pig feed. As can be observed from the plasma profiles, absorption occurs quite rapidly, with no significant lag time observed for any of the feeding regimens. There appears to be a slight trend towards lower overall absorption in both the fed state regimens, though as with the fenofibrate leg of the current study, the limited numbers prohibit tests of statistical significance.
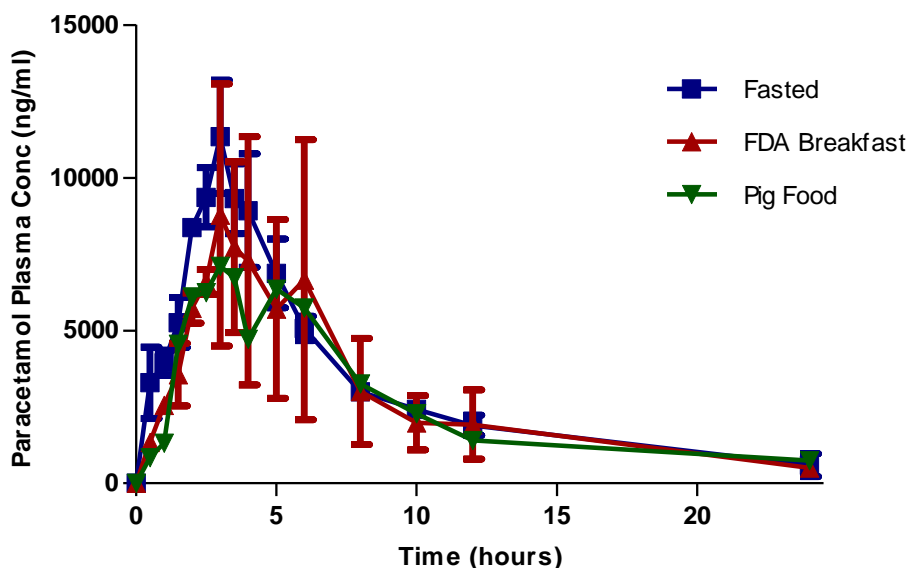
Figure 5-4 Plasma concentration of paracetamol vs. time profiles after oral administration of Paralief® 500mg tablets, (■) indicates fasted state (n=3, mean ± range), (▲) indicates fed half FDA standard breakfast (n=2, mean ± range), (●) indicates fed 175g weanling pellet feed (n=1).

Figure 5-5 and table 5-3 summarise the oral pharmacokinetics of paracetamol after oral administration of Paralief® 500mg tablets in the fasted and in both fed states. There appears to be a slight trend towards lower overall absorption of paracetamol in the fed studies. This appears to be more substantial with regard to C_{max} rather than overall AUC. This is a trend often observed for immediate release preparations in the fed state, where a reduced C_{max} is observed due to reduced absorption rate, indicating some promise in the ability of the pig food effect model to discriminate between fasted and fed performance for BCS class I compounds. However, this is not corroborated by a prolonged T_{max}, with all values lying in the range of 2-3 hours for all pigs in all treatments. While longer than the typical gastric emptying rate of less

than 30 minutes in fasted humans, the gastric emptying rate observed in this study in landrace pigs appears to be quicker and less variable than that previously reported (Sjogren et al., 2014, Davis et al., 2001). Of particular interest is that the delayed absorption of fenofibrate observed when administered with pig food (figure 5-2) is not replicated in the absorption of paracetamol and T_{max} has not been affected by feeding standard pig pellet feed, as had been observed for fenofibrate.
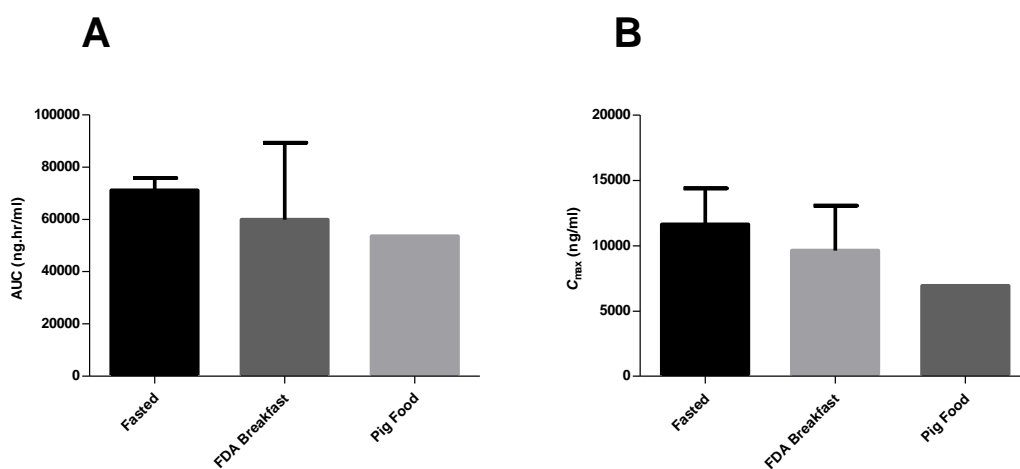

Figure 5-5 Bioavailability at 24 hours (A) and C_{max} (B) of paracetamol from Paralief® 500mg tablets in fasted (n=3, mean ± range), FDA breakfast fed pigs (n=2, mean ± range) and following 175g weanling pellet feed (n=1)

Table 5-3 Summary of pharmacokinetic parameters after oral administration of paracetamol as Paralief® 500mg tablets to fasted pigs (n=3, mean (range)), and to pigs fed FDA breakfast (n = 2, mean (range)) or 175g weanling pellet feed (n=1)

[a] median (range)

	Oral pharmacokinetic parameters		
	Fasted	**FDA Breakfast**	**Pig Food**
C_{max} **(ng/mL)**	11449 (8281 – 14331)	9646 (6226 – 13066)	7110
T_{max} **(hours)[a]**	3 (2-3)	2.5 (2-3)	3
$AUC_{0 \to 24hrs}$ **(ng.h/ml)**	74248 (68042 – 80353)	65735 (36343 – 95127)	60375

Effect of feeding regimen on gastric emptying

In order to assess the effect of the varying fasting regimens, and to compare the characteristics of both fasted and fed state gastrointestinal contents post mortem sampling of the gastrointestinal contents was performed. Based on results of previous studies where a 16 hour fast was insufficient to ensure complete emptying of gastric contents, the ability of various fasting regimens to generate a truly 'fasted state' was assessed. Results of post-mortem assessment of pig gastrointestinal contents are summarised in table 5-4 and visualised in figure 5-6. Generating a fed state by providing daily food requirement as a single feed prior to euthanasia and sampling of contents led to a large increase in both solid and liquid content in both the stomach and small intestine (4 hour b.d.), which was accompanied with somewhat of a buffering effect on gastric pH. The rise of gastric pH, however, is perhaps not as substantial as would be predicted, given the volume of food present in the stomach.

It is the effect of different fasting regimens on the day prior to euthanasia, however, which are most notable. Despite being last fasted for almost 24 hours, feeding a large volume of pellet feed the day before analysis led to significant volumes of fluid and solid content in both the stomach and small intestine (24 hour o.d.). The analysis also revealed a high level of variability, with two of the five animals displaying virtually empty stomachs, while up to 199g of solid content was observed in one animal. There was also a noticeable trend for pH increases with increasing solid content most likely due to the buffering effect of remaining food material.

In order to ensure a completely fasted state was achieved prior to dosing in future studies a range of other fasting regimens were investigated. Feeding regimens consisting of two smaller meals, rather than one large meal were trialled, involving feeding 175g of pellet feed twice daily. The reduced quantity of food fed 24 hours prior to sampling appears to be sufficient to induce a fasted state, with only very small levels of solid content observed in the stomach (max 3.09g) and small intestine (max 6.13g) (24 hour b.d.). When the final feed was given 16 hours prior to sampling there appears to be more inconsistency with regard to the presence of remaining food, with up to 60g of solid content observed in the stomach, and 94g in the small intestine, indicating that a 16 hour fast is insufficient to induce a complete fasted state (16 hour b.d.). However, no significant effects were determined between the feeding regimens as the current study was not sufficiently powered to observe a significant effect considering the variability in gastrointestinal contents.

181

Table 5-4 Post-mortem assessment of pig gastrointestinal contents; all parameters reported as median (range)

24 hour o.d. (n=5); 500g pellet feed once daily; last feed 24 hours prior to euthanasia

4 hour b.d. (n=3) 175g pellet feed twice daily; fed 500g pellet feed 4 hours prior to euthanasia

16 hour b.d. (n=3) 175g pellet feed twice daily; last feed 16 hours prior to euthanasia

24 hour b.d. (n=6), 175g pellet feed twice daily; last feed 24 hours prior to euthanasia

Feeding regimen	Stomach			Small Intestine		
	Fluid Volume (ml)	Solid Content (g)	pH	Fluid Volume (ml)	Solid Content (g)	pH
24 hour o.d.	56 (30–220)	22.47 (2.58–199.26)	2.25 (2 – 4.79)	99 (34 – 156)	72.39 (13.66 – 102.9)	7.26 (7 – 7.52)
4 hour b.d.	221 (85–269)	357.91 (191.83–446.55)	3.44 (3.14 – 3.91)	351 (127 – 454)	213.15 (174.93 – 279.76)	5.9 (5.26 – 6.73)
16 hour b.d.	43 (30–88)	9.00 (4.10–59.91)	3.1 (1.81 – 3.4)	185 (130 – 245)	10.56 (10.26 – 93.88)	6.31 (6.29 – 6.75)
24 hour b.d.	29.5 (23–55)	1.76 (0.96–3.09)	2.205 (2.05 – 3.4)	93 (41 – 168)	4.005 (1.31 – 6.13)	7.68 (7.41 – 7.81)
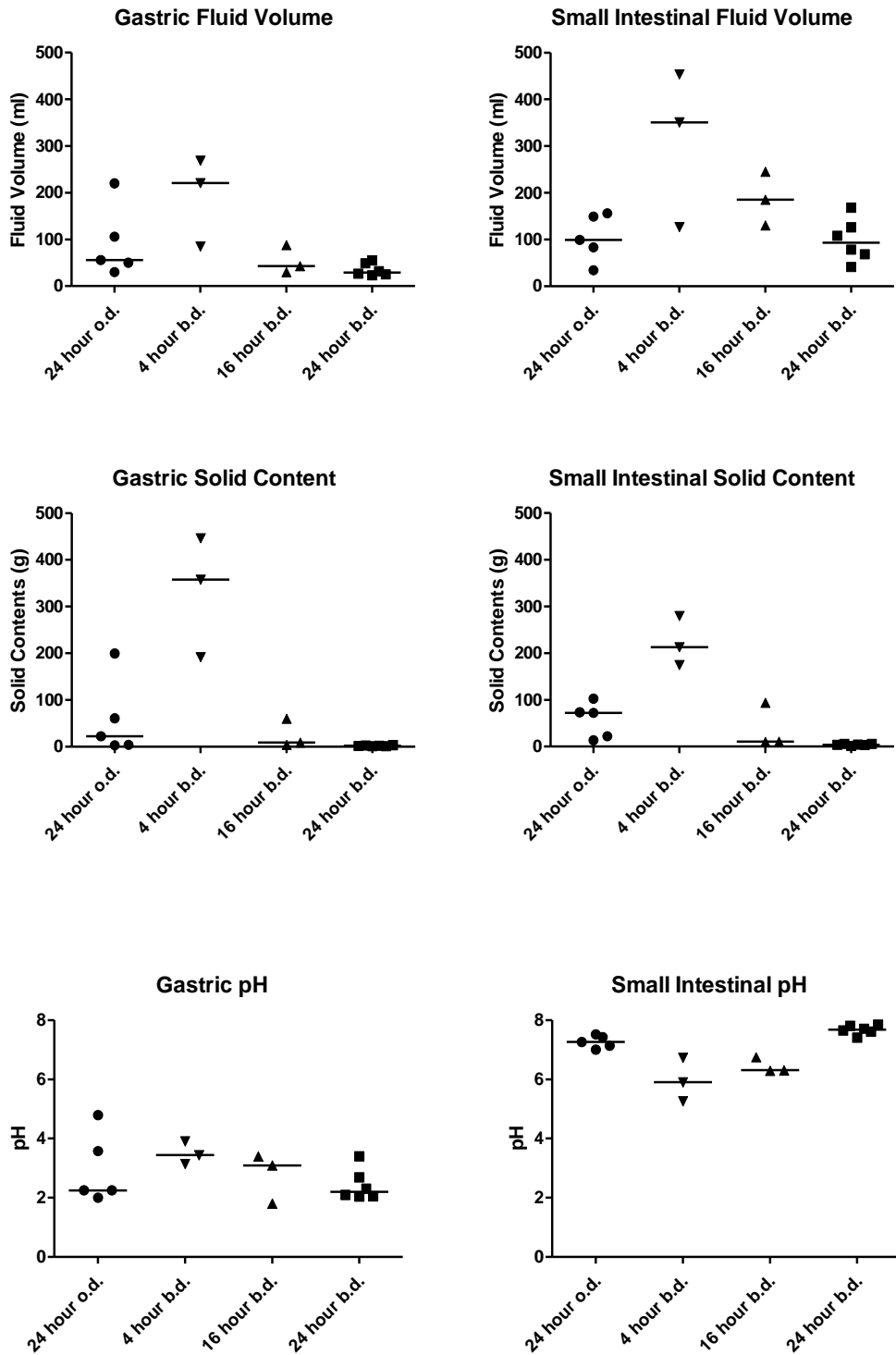
Figure 5-6 Scatter dot plot of post-mortem assessment of pig gastrointestinal contents; line at median

24 hour o.d. (n=5); 500g pellet feed once daily; last feed 24 hours prior to euthanasia

4 hour b.d. (n=3) 175g pellet feed twice daily; fed 500g pellet 4 hours prior to euthanasia

16 hour b.d. (n=3) 175g pellet feed twice daily; last feed 16 hours prior to euthanasia

24 hour b.d. (n=6), 175g pellet feed twice daily; last feed 24 hours prior to euthanasia

Discussion

Effect of food on fenofibrate bioavailability in pigs

The primary aim of the current work was to evaluate the utility of the pig model to predict food effects on oral bioavailability *in vivo*. To date, the most commonly used preclinical food effect model has been the dog, and Lentz *et al.* (2006) have developed a food effect model in beagle dogs based on feeding a 50g aliquot of a homogenised FDA high-fat meal which provided a close qualitative agreement to human data. However, given that canine gastric pH is considered to be, on average, higher and more variable than humans, dogs must be pre-treated with pentagastrin to simulate the lower gastric pH conditions of humans. In their study, Lentz *et al.* demonstrated that both the type and quantity of food are critical, with a specialised high-fat dog meal proving ineffective in inducing a positive food effect and 100g high-fat FDA meal over-predicting the human food effect. This model has been further used and adapted, proving useful in assessing food effect *in vivo*, with greater success in qualitative rather than quantitative predictions and a general trend towards over-prediction of human food effects in dog models (Mathias et al., 2015, Zane et al., 2014).

With the growing popularity of the pig as a pre-clinical animal model, Christiansen *et al.* subsequently adapted this food-effect protocol for use in Göttingen minipigs (Christiansen et al., 2015). In their study, Christiansen *et al.* investigated the effects of both pentagastrin pre-treatment and the type and amount of food on *in vivo* food effects in Göttingen minipigs. However, no significant food effects were observed for compounds exhibiting either a positive (atazanavir) or negative (pravastatin) food

184

effect in their study. It is in the context of the analysis of this study that the current protocol was designed and investigated.

In this previous study, the effects of feeding a homogenised FDA breakfast at two different volumes, 5g/kg bodyweight and 10g/kg bodyweight, as well as the potential for replacing the food with Fresubin® liquid energy drink. A key outcome from this study was to suggest that in order to improve the function of a pig model of food effect there was both a need for higher caloric and more viscous meals, while also persisting with the FDA breakfast as a food source due to its tendency to predict a positive food effect for atazanavir (Christiansen et al., 2015). Therefore, in the current study the design for assessment of positive food effect in pigs it was decided to use the FDA meal in increased quantities in landrace pigs. The current study uses a fixed volume of food, at half the volume used in human food effect studies, as described in table 5-1. This results in an approximate doubling of food relative to previous studies in minipigs (mean 19g food /kg bodyweight; range 18.2 – 20.1g) with the intention of maximising the potential for observing positive food effects. The effect of pig food on bioavailability was also assessed, and while the rate of absorption of fenofibrate appeared to be reduced somewhat, detailed analysis of the data was not feasible in this case, as the highest observed plasma concentration occurred at the T_{last}, meaning determination of AUC, C_{max} or T_{max} was not possible. Interestingly a similar observation has been made in a food effect study in mini-pigs using atazanavir as a model compound for positive food effect, with the final (24hr) time-point corresponding to T_{max} for at least one animal in each of a range dosing regimens (Christiansen et al., 2015).

185

Fenofibrate displays a formulation dependent, positive food effect in humans, with approximately 35% - 50% increase in bioavailability from Lipantil® Micro in humans in the fed state, dependent on composition of a meal, and in particular the fat content, with even greater increases in C_{max} (Guivarc'h et al., 2004, Guichard and Sauron, 1993, Ling et al., 2013). The current studies main aim was to assess whether this can be emulated in a pig food effect model. As can be seen from the results, in figures 5-2 and 5-3 and table 5-2, there was no significant difference in food effect between the fasted and FDA breakfast fed legs of the current study, and in fact with a trend towards a negative food effect in the fed state. It appears that FDA breakfast fed pigs do not demonstrate solubility and dissolution enhancing effects that promote absorption of fenofibrate. Further characterisation is required to assess the physiological and physicochemical environment in the fed state pig, and in particular the effects of different meal composition on the post-prandial pig gastrointestinal environment. Specifically, the effect of varying quantities of an FDA high fat breakfast on the gastrointestinal environment will provide an insight into the lack of a food effect observed both here and previously (Christiansen et al., 2015). It seems plausible, based on the lack of an observed food effect on the extent of fenofibrate absorption and on gastric emptying, that the current meal caloric content and volume is too small to induce a response.

A comparison of the food effect between humans and in pigs, as described by the fold differences in bioavailability in the fed and fasted state, is presented in figure 5-7. There is a poor correlation between fenofibrate food effects in humans and pigs. The results in the current study further demonstrate the limitations of a pig model of

food effect, as despite the increased food volume relative to previous studies, no

significant food effects were demonstrated.
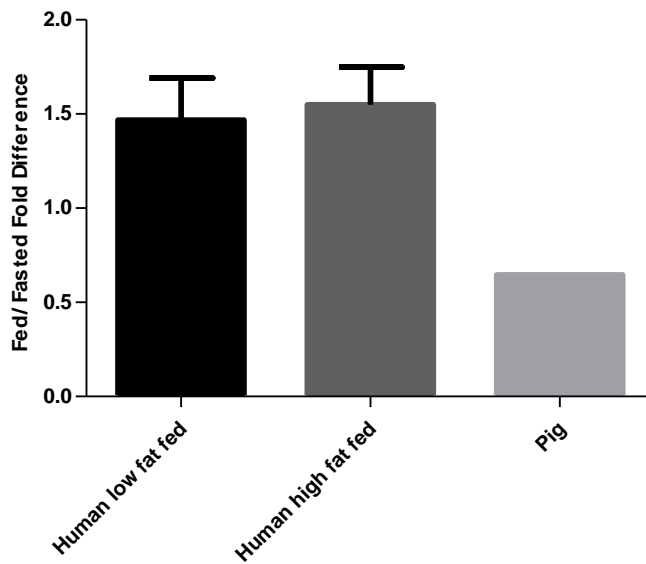

Figure 5-7 Lipantil® Micro food effect expressed as fold difference in AUC_{fed}/AUC_{fasted}

Human low fat fed extracted from (Guivarc'h et al., 2004)

Human high fat fed extracted from (Guichard and Sauron, 1993)

Effect of food on paracetamol absorption and gastric emptying

A secondary objective of the current study was assessment of gastric emptying in fed and fasted pigs, using paracetamol as a marker. Henze *et al.* (2018a) and Christiansen *et al.* (2015) have previously used this approach to address the general disagreement in the literature regarding the gastric emptying rate in pigs, and the effect of food on gastric emptying. While in their studies, Göttingen minipigs rather than Landrace pigs were used, a similar trend to the current study, where no significant changes in paracetamol T_{max} was observed between fasted and fed states. Gastric emptying is anticipated to slow post-prandially, with larger effects seen with increasing caloric density and increasing volume of ingested food (Abuhelwa et al., 2016b, Hunt and Stubbs, 1975). In the current study, there is no significant differences in the gastric emptying rates in pigs fed the FDA breakfast, nor with the pellet feed, as assessed by paracetamol T_{max}. The gastric transit observed in fasted pigs (2-3 hours) in the current study is longer than that observed in humans in the fasted state of less than 0.5 hours (Sjogren et al., 2014, Abuhelwa et al., 2016b). Though the current sample size is small, there is less variation in gastric emptying than has previously been reported in pigs (Christiansen et al., 2015, Suenderhauf et al., 2014, Suenderhauf and Parrott, 2013, Oberle and Das, 1994, Davis et al., 2001, Henze et al., 2018a).

This longer gastric transit in pigs relative to that in humans may contribute to the delayed T_{max} observed for fenofibrate (6 – 10 hours) in this and previous studies, compared to clinical, human data (Guivarc'h et al., 2004, O'Shea et al., 2015). However, this does not fully account for the delayed and reduced absorption of fenofibrate when administered with pellet feed. Despite similar gastric emptying rates being observed for paracetamol in pellet fed pigs relative to fasted and FDA

188

breakfast fed groups, there is substantial changes in fenofibrate absorption, with appreciable levels of absorption only observed at 24 hours, which corresponds to T_{last} of the current study. Thus, it appears that drug and/or formulation dependent inhibition of gastrointestinal absorptive and/or transit processes are observed in pellet fed pigs. The rate and extent of fenofibrate absorption in pellet fed pigs is significantly different to that observed in pigs fed the FDA breakfast, while absorption of paracetamol in these two groups appears to be quite consistent. This emphasizes the importance of having completely fasted pigs in fasted state studies, and also demonstrates the limitations of implementing a food effect study utilising standard pig feed as formulation dependent variation can occur. A similar limitation was observed when utilising a dog specific high fat diet, and supports the continued use of an FDA breakfast, or similar, in pre-clinical food effect models (Lentz et al., 2007, Christiansen et al., 2015).

Effect of feeding regimen on gastrointestinal contents

An area of particular concern and a significant limitation of pig models of absorption, particularly with regard to fasted state bioavailability studies, which has become apparent in recent years is the current inability to ensure a true fasted state prior to study commencement (Christiansen et al., 2015, Suenderhauf et al., 2014). Recently a potential limitation of the utility of the pig, and specifically the minipig, in assessment of bioavailability has been identified as that of incomplete food emptying depending on the fasting protocol utilised (Suenderhauf et al., 2014). This has been suggested to be one potential cause for the longer gastric transit time observed, relative to humans. In their study, Suenderhauf *et al.* (2014) observed that despite a 12 hour fast, pigs fed a pellet diet the day before dosing retained a significant amount of food in the stomach. This is a potential limitation where a fasted study protocol is required, and was overcome by feeding a liquid meal the day before dosing or by pre-treating with i.m. metoclopramide, a pro-kinetic agent, though such a pharmacological intervention may limit the applicability of a model designed to investigate the effect of food on absorption, where altered gastric transit is a significant variable of interest (Suenderhauf et al., 2014).

A similar concern arose rather serendipitously during the conduct of the studies outlined in the current thesis, when during a post-mortem examination of a 'fasted' pig, the stomach was found to contain a substantial volume of solid material. This led to the design of the current study to assess the effects of different fasting regimens on stomach contents. As with the previous analysis carried out by Suenderhauf *et al.* (2013), it was observed that even a fast of up to 20 hours was insufficient to ensure complete emptying of gastric contents when a large feed volume is given (24 hour

190

o.d.; figure 5-6). It appears that the emptying is inherently variable and complete emptying cannot be assured. Anecdotal observations of feeding habits pointed to a tendency for animals to gorge on the available feed volume before becoming sedentary and docile post-prandially. This led to the suggestion to restrict the feed to a smaller volume twice daily, with the aim to keep pigs more mobile, promoting gastric motility, and potentially stimulating the gastroileal reflex and promoting gastrointestinal transit. In this regimen the final feed was given 24 hours prior to dosing. When adopting this feeding regimen, all animals analysed post mortem had insignificant levels of solid content in either the stomach or small intestine, with a trend towards reduced stomach fluid content and similar intestinal fluid content. There also appeared to be a trend toward increased stomach acidity, with a corresponding increase in small intestinal pH (24 hour b.d.; figure 5-6). All this data correlates well with the expected result when moving from a 'fed' to a 'fasted' state, suggesting the modified feeding regimen was adequate to ensure a completely fasted state.

The twice-daily feeding regimen was further investigated, to assess whether it was the multiple daily feedings or the reduced food provided on the day prior to dosing that ensured a completely fasted state. In this regimen, a second feed on the day prior to analysis was provided 16 hours prior to euthanasia. Of the three pigs analysed, one demonstrated appreciable levels of solid material in both the stomach and small intestine (16 hour b.d.; figure 5-6), suggesting that fasting cannot be ensured when feeding the evening prior to dosing.

191

Finally, pigs were analysed in a fed state to compare the stomach contents to that in the original feeding regimen in order to assess how closely the original 'fasted' state resembled a true fed state (4 hour b.d., figure 5-6). Unsurprisingly, there is a far greater volume of both solid and liquid intestinal contents in the fed pigs. However, it is noteworthy that the upper limits of the 'fasted' regimen are comparable to the lower limits of the fed regimen, suggesting that, in at least some instances, what was thought to be fasted pig may at least have been in a semi-prandial state. The variability in gastric contents, in particular, has the potential to cause variable gastrointestinal transit and absorption. Thus, it appears necessary to carefully design, not only the fed state aspect of a food effect study in pigs, but due consideration must also be given to ensure adequate fasting.

Conclusion

In conclusion, the current study demonstrates continued limitations in the assessment of food effect in the pig model. Despite attempting to address some of the issues previously encountered in similar studies, the current protocol is not effective at inducing a food effect for fenofibrate. It appears that the current meal volume and caloric content is insufficient to cause an increase in solubilisation. This is further supported by the lack of a delayed gastric transit in fed pigs, as demonstrated by the similar T_{max} and C_{max} values for paracetamol in the fasted and fed state. Further optimisation of this model is necessary in order to use pigs to predict food effects in humans. Specifically, the effects of varying fed regimens with regard to the meal composition and quantity, the characterisation of pig gastrointestinal physiology, in particular gastric transit, and the physicochemical properties of intestinal contents with varying quantities of food are priorities. These measures will be useful in providing a mechanistic understanding for the lack of an observed food effect in the current and similar studies.

The current study does, however, provide useful insights into definition of effective fasting protocols. This study has demonstrated that the presence of food leads to unpredictable responses in drug absorption in pigs, therefore it is critical to ensure that all fasted studies in pigs are appropriately defined. The data presented here demonstrate that that feeding smaller volumes twice daily and providing a final feed 24 hours prior to dosing is an effective approach to ensure a fasted state. The implementation of such a fasting regimen is crucial to the performance of future pig models of bioavailability and food-effect.

Chapter 6 : General discussion

Overview and Summary

It is widely recognised that R&D productivity in terms of bringing new licensed medicines to market has consistently decreased, with the number of new medicines approved per $1 billion R&D invested decreasing by 50% every nine years since the 1950s (Scannell et al., 2012). One of the key challenges in the development of new oral medicines is that most emerging drug molecules display poor solubility, leading to poor oral bioavailability. The poor intrinsic solubility of Biopharmaceutical Classification Scheme (BCS) class II compounds has stifled development of many emerging therapeutic compounds. With up to 75% of drug development candidates displaying poor aqueous solubility, the bioavailability limitations posed still form an unmet challenge for pharmaceutical drug development (Di et al., 2012). The absorption of these poorly water soluble drugs (PWSD) is limited by their poor solubility and resultant slow dissolution rate within gastrointestinal fluid (Butler and Dressman, 2010). With the ever increasing prevalence of lipophilic, poorly soluble compounds in drug development pipelines, the identification of compounds with optimized pharmacodynamic properties, but poor 'developability' owing to sub-optimal absorption properties leads to formulation and delivery challenges in drug development, where significant delays or even failure to gain approval can occur (Butler and Dressman, 2010, Hauss, 2007). The need to develop novel bioenabling formulation technology tailored to the properties of the drug molecule is, therefore, critical.

A key issue is that there has traditionally been a lot of 'trial and error' involved in formulation screening, with a series of formulations assessed in preclinical models,

with a view to identifying the optimal candidate for Phase 1 clinical trials. This conventional formulation development approach consists of initial *in vitro* screening, followed by pre-clinical bioavailability studies and subsequent clinical evaluation in humans. Traditionally, this process has proven both iterative and laborious, as *in vitro* and pre-clinical screening remained largely empiric, rather than mechanistically driven. This has led to significant wastage in drug development, where initial screening has been a poor indicator of clinical performance and this has been a contributory factor to the growing cost of drug development. This approach leads to excessive *in vivo* bioavailability testing in animals, and possibly a range of animals, generally as a result of gaps in the knowledge regarding which choice of animal model is most appropriate for the drug and formulation under investigation and poor understanding of their biopharmaceutical properties. There is consequently a need to increase R&D productivity by expediting development of new drug molecules to facilitate earlier access to patients. A key tenet of this process is improving the ability to select formulation approaches with the highest potential for success, and screen out those unlikely to succeed earlier in the drug development process, ensuring a 'quick win, fast fail' for developers (Paul et al., 2010).

In light of these development bottlenecks, a key theme in modern drug and formulation development is to move from a "test and confirm" to a "learn and confirm" paradigm, where predictions will be made in the 'learn phase' on how to maximise medical value for a new drug in advance of *in vivo* studies. In this scenario, *in vivo* studies become 'confirmatory' rather than 'exploratory' (Selen et al., 2014). Adopting a 'learn and confirm' approach for development of bioenabling formulations could prove beneficial both with regard to improving the screening

196

capability of early *in vitro* tests and in validating the predictive capacity of pre-clinical models in respect of clinical biopharmaceutics. Improving the predictive capabilities of *in vitro* screening tools consists of two main approaches; firstly improving the biorelevance of the test itself, through the utilisation of approaches such as simulated intestinal media, by adapting equipment to more accurately physiological processes, such as passage through the GIT or hydrodynamic processes, or by adding a dynamic aspect to the screening tool, such as introducing an absorption sink, inducing lipolysis or by utilising a transfer model approach; or secondly by integrating *in vitro* screening results into predictive models utilising *in silico* approaches. The range of *in silico* approaches is many and varied and can either be empiric or mechanistic in approach. The choice of approach will be dictated both by availability of data and expertise and also by the desired level of mechanistic understanding.

Considering the limitations of current R&D approaches outlined above, and recognizing that most new drugs are poorly soluble, the drive to accelerate the development and approval of break-through therapy drugs urgently requires an accelerated development paradigm, consisting of three key elements:

1. Design of innovative, bioenabling formulations for poorly soluble drugs, with choice guided through knowledge harnessed in formulation screening

2. Generation of predictive *in vitro* and *in silico* tools capable of rapidly and accurately screening formulations and predicting their ability to deliver drug *in vivo*

3. Improve the predictive capacity of pre-clinical *in vivo* testing by prudent choice of animal model and understanding of the key biopharmaceutical properties of model choice

In light of the factors described here, the specific objectives of this thesis are, therefore, to assess novel bioenabling formulations and new *in vitro* and *in silico* tools to predict their *in vivo* performance in pigs as a means to improve efficiency in formulation development. The current thesis aims to shed new insights on the processes involved in drug product development.

Specifically, we have assessed the utility of the pig as a pre-clinical animal model with regard to the assessment of bioenabling approaches, using dissolution enhancing formulations and by concomitantly administering dosage forms with food, placing specific emphasis on eliminating food mediated changes in bioavailability. The ability of biorelevant screening approaches, in conjunction with *in silico* approaches of varying levels of complexity to predict *in vivo* performance and the potential of the pig to act as a model for food effect bioavailability were also assessed. The approaches involved in assessing these aims have been described in detail in the preceding chapters, and an overall, general discussion of the findings and how they relate to the wider literature is provided here.

Bioenabling approaches in drug development

With the increasing proportion of drug candidates with relatively poor biopharmaceutical properties, the drive to design novel "bioenabling formulations", to enhance oral absorption has increasingly gained attention. As a result, biopharmaceutical assessment is shifting focus from drug candidate-assessment to formulation assessment (Buckley et al., 2013). While numerous formulation technology platforms have been developed with the aim of improving the bioavailability of poorly soluble drugs, the simplified hypothesis behind each design is the ability to make a BCS class II compound behave like a BCS class I compound (Kawabata et al., 2011). This is achieved through maximising dissolution and solubilisation of drug in the GIT through the use of a range of different formulations including lipid based formulations, amorphous solid dispersions and mesoporous carriers, approaches considered in this thesis (Williams et al., 2013b, Bergström et al., 2016). However, despite this increased interest, significant improvements in bioavailability *in vivo* through the use of bioenabling formulations remains challenging, as demonstrated by the relatively low number of successful commercial examples. While there has been a wide range of formulations proposed and evaluated *in vitro*, the lack of direct *in vitro* to *in vivo* correlations means that the significant increases in dissolution/ solubilisation enhancement *in vitro* rarely translate to comparable increases in *in vivo* bioavailability (Buckley et al., 2013).

Accordingly, a primary aim of the current thesis was to investigate the bioenabling potential of a variety of novel formulations in a large animal model, in this case, the pig. Thus, two novel fenofibrate containing formulation platforms have successfully

been assessed in fasted pigs. Fenofibrate was chosen as a novel compound as its absorption and bioavailability have been well characterised and is typical of a BCS class II compound, with conventional formulations of fenofibrate displaying low and variable bioavailability in the fasted state due to low solubility and resultant slow dissolution (Miller and Spence, 1998). Fenofibrate bioavailability increases significantly with increased solubilisation and/or dissolution, as demonstrated by the considerable increases in absorption when delivered either in the fed state or in dissolution enhancing formulations (Sauron et al., 2006, Ling et al., 2013). Thus, it is seen as a reliable model for assessment of the formulation approaches chosen. In order to facilitate comparison, both a crude reference formulation of fenofibrate and a commercial micronised formulation were also delivered. A comparison of the pharmacokinetic parameters of all administered formulations are summarised in figure 6-1 below, while figure 6-2 provides a snapshot of the plasma concentrations from all the trials conducted.

As can be seen from the data presented here, both mesoporous silica based formulations and the lipidic dispersion formulation improved both the rate and extent of bioavailability of fenofibrate, relative to the crude formulation, to a similar or greater extent compared to the commercial Lipantil® Micro formulation. Thus, the potential for two novel bioenabling formulations to enhance the oral bioavailability of poorly water soluble drugs, using fenofibrate as a model compound, has been demonstrated.

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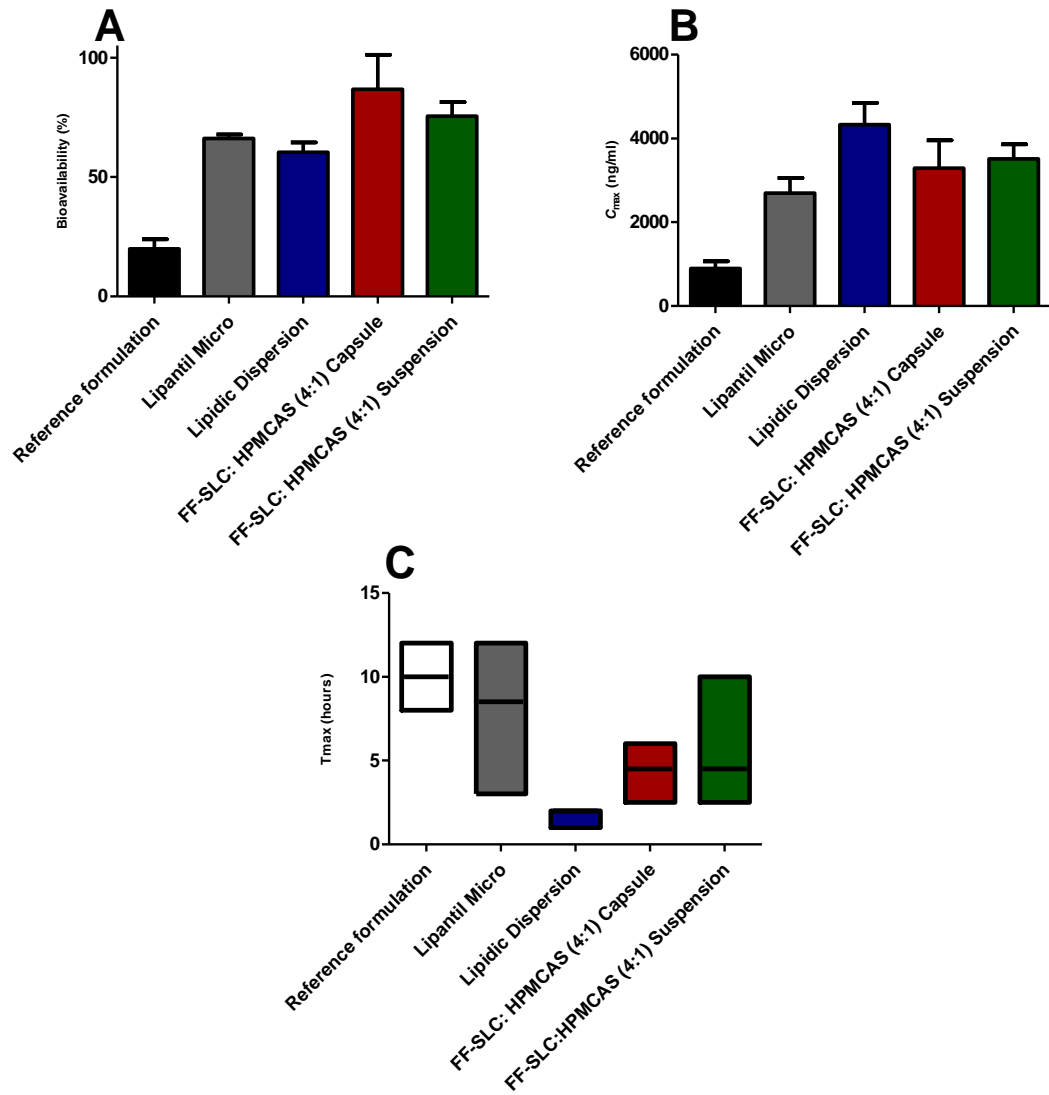
Figure 6-1 Summary pharmacokinetics of fenofibric acid following oral delivery of 67mg fenofibrate from reference formulation (n = 6), Lipantil® Micro (n = 4), lipidic dispersion (n = 4), FF-SLC: HPMCAS (4:1) capsules (n =6) and FF-SLC: HPMCAS (4:1) suspension (n=6). (a) Displays bioavailability, (mean ± SD) (b) displays C_{max} (mean ± SD), and (c) displays T_{max} (median, range).
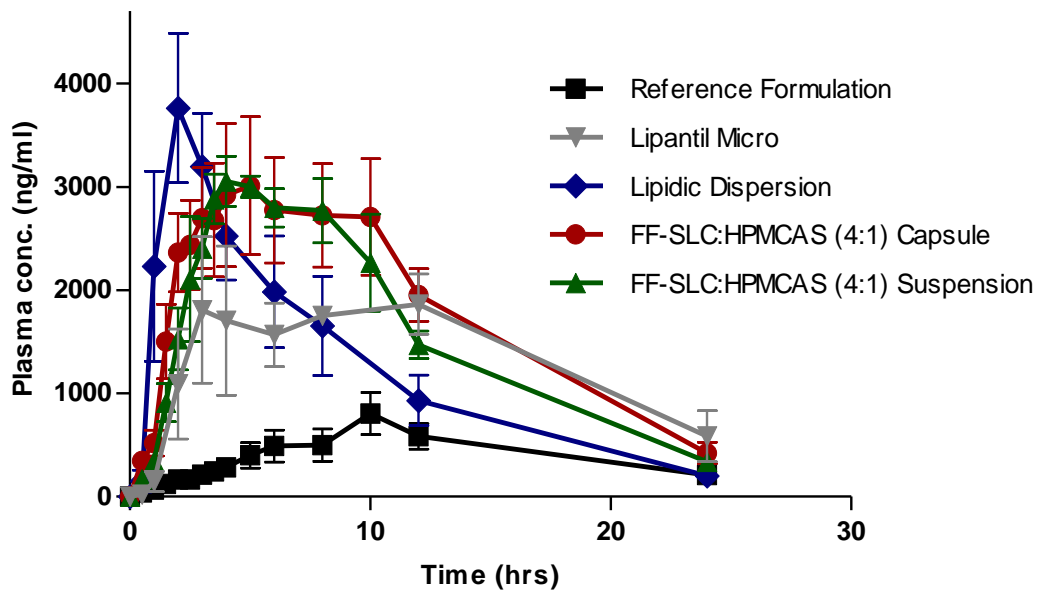
Figure 6-2 Plasma concentration of fenofibric acid vs. time profiles after oral administration of 67 mg to fasted pig, all data mean ± SE, ■ Reference formulation (n=6), ▼ Lipantil Micro (n=4), ◆ Lipidic Dispersion (n=4), ● FF-SLC : HPMCAS (4: 1) capsules (n=6) and ▲ FF-SLC : HPMCAS (4: 1) suspension (n=6)

Mesoporous silica and precipitation inhibitors

Mesoporous silicas have been the focus of a significant amount of research in recent years with regard to enhancing absorption and bioavailability of poorly water soluble drugs (Maleki et al., 2017, Riikonen et al., 2018). Traditionally, mesoporous materials have been loaded with poorly soluble drugs that exist as molecularly adsorbed with the aim of improving drug dissolution and, hence, bioavailability through the generation of supersaturation (McCarthy et al., 2016). Numerous *in vivo* studies in both rodent and large animal models have demonstrated significant improvements in bioavailability (Van Speybroeck et al., 2010a, Van Speybroeck et al., 2011, Kiekens et al., 2012, Bukara et al., 2016a). While recently, a study utilising fenofibrate loaded mesoporous silica was the first to demonstrate proof of this concept in man, further emphasizing the potential of mesoporous silica formulations (Bukara et al., 2016b). However, a potential problem with generation of supersaturation in mesoporous silica based formulations is the potential to cause precipitation, and for this reason recent focus has turned to the co-administration of precipitation inhibitors with these silica formulations (Dressman et al., 2016, McCarthy et al., 2016). To date, a number of pre-clinical studies using such formulations have been carried out in rodents (Laine et al., 2016, Van Speybroeck et al., 2010b). To our knowledge, the bioavailability study outlined in chapter two, with results summarised in figures 6-1 and 6-2, is the first large animal study to utilise such an approach. In this study both the rate and extent of bioavailability were significantly increased by delivery of the fenofibrate-loaded silica with HPMCAS, relative to the crude fenofibrate formulation, with bioavailability comparing favourably to previously assessed formulations in fasted pigs.

Lipidic dispersion: a solid lipid based formulation

Similarly, lipid based formulations (LBF), and in particular self-emulsifying drug delivery systems (SEDDs), have long been investigated for their potential to improve bioavailability of PWSD, with numerous commercial examples. Fenofibrate also has a long history as a model compound for the assessment of LBF, with numerous preclinical studies demonstrating improvement in fenofibrate bioavailability from such formulations (Griffin et al., 2014, Thomas et al., 2014) and prototype fenofibrate containing LBF have also been demonstrated to enhance bioavailability in fasted humans (Fei et al., 2013). While traditionally LBF have consisted of liquid or semi-solid formulations, significant research has been focussed on methods to generate solidified LBF, owing to industrial preference for solid forms, mainly due to cost and capsule compatibility issues (Riikonen et al., 2018, Jannin et al., 2008, Tan et al., 2013). The bioavailability study carried out as part of chapter three investigates the use of solidified LBF through combination of a SEDDs with traditional solid dispersion technology aims to generate a novel "third-generation" solid dispersion (Vo et al., 2013). The combination of lipid mediated solubilisation, along with altered solid-state characteristics of the drug enhanced dissolution, both *in vitro* and *in vivo* with resultant improvement in the rate of drug absorption. This improvement in dissolution was also predicted to eliminate food-mediated alterations in bioavailability.

In summary, two novel approaches enabling approaches have been assessed in the fasted pig model and have demonstrated positive results with regard to improving bioavailability. This lends further support to the growing case for clinical assessment of both solidified LBF and mesoporous silica formulations.

The pig as a pre-clinical animal model

Large animal models represent a costly yet valuable tool in the evaluation of the gastrointestinal absorption of oral dosage forms. With increasing focus on the predictive capacity of animal models and on the scientific justifications and ethical implications of animal testing, there has been renewed interest in the use of the pig model (Forster et al., 2010, Bode et al., 2010, Colleton et al., 2016). In recent years, the pig has become increasingly popular in pre-clinical bioavailability studies owing to the perceived similarities in gastrointestinal anatomy and physiology between pigs and humans (Walters et al., 2011, Puccinelli et al., 2011, Suenderhauf and Parrott, 2013, Sjogren et al., 2014, Swindle, 2016, Swindle and Smith, 1998, Davis et al., 2001). Pigs, like humans, are omnivorous (Patterson et al., 2008), they have similar digestive system (Davis et al., 2001, Kararli, 1995) and they are a suitable model for evaluation of many biopharmaceutical aspects of drug absorption, distribution, metabolism and elimination (van der Laan et al., 2010). While there are numerous drug delivery technologies designed to enhance oral bioavailability, it is increasingly clear that each formulation platform cannot be universally applied to all poorly water-soluble drugs and the selection of the correct formulation technology is often complex. As a result, it is crucial that the pre-clinical development program provides an accurate performance assessment and a key question that needs to be addressed in pre-clinical evaluation is whether the chosen animal model will reliably predict the *in vivo* performance of a selected drug and/or formulation technology. With the increasing focus on the pig in pre-clinical development programs, this thesis poses three general questions;

205

1. Does bioavailability in the pig accurately reflect bioavailability in humans?

2. Does the pig model discriminate in screening of bioenabling formulations?

3. Can a pig food effect model successfully predict human food effect?

The pig as a predictor of human bioavailability

Henze *et al.* (2018b) have recently investigated the correlation between pig and human bioavailability by conducting a systematic review of studies involving compounds for which absolute bioavailability was available. This analysis included data from various pig breeds, namely Landrace, Yucatan and Gottingen minipig, and found a moderate positive correlation (r=0.4985, p = 0.0253) for the 20 compounds identified, a correlation similar to that of the canine model. There are, however, cases where the bioavailability of specific compounds varies significantly between humans and pigs, particularly in cases where specific metabolising enzymes appear to lack homologues between species. The authors identified metoprolol and diclofenac as particular cases where differences in rates and extent of metabolism in pigs may differ to that in humans, particularly with regard to the effect of first pass metabolism (Henze et al., 2018b). Assessment of the absolute oral bioavailability of celecoxib in pigs in chapter four further supports this hypothesis, where the extent of oral bioavailability was quite variable, while such high variability was not reflected in intravenous administration. This has been observed in previous studies comparing oral and parenteral drug delivery in pigs, such as that carried out by Holm *et al.* (2013). In their study buccal administration of metoprolol was examined and large increases in absorption and reduction in variability were observed relative to oral delivery, indicating that differences in first pass metabolism may play a significant role in differences in oral bioavailability between pigs and humans (Holm et al.,

2013a). Similarly, parenteral administration of celecoxib in this study (*via* the intravenous route in this case) reduced the variability in exposure relative to the oral route. Overall, while there appears to be a moderate correlation between pig and human bioavailability, prudent selection of model compounds may improve the predictive capacity. Particular consideration should be given to the enzymes responsible for drug metabolism, and the occurrence of their homologues in pigs. The data presented in chapter four lend further support to this hypothesis and emphasize the desire for improved characterisation of the cytochrome P450 isozymes present in pigs. The primary aim of chapter four of this thesis was originally the assessment of the absolute oral bioavailability of celecoxib pigs in order to assess its utility as a model compound for the assessment of bioenabling formulations, along with its potential to act as a "borderline" case in a food-effect model. Overall, the results of the study suggest that celecoxib would not be an appropriate model compound for such studies, however analysis of the extent and variability of celecoxib bioavailability in pigs has provided useful insights into the pig as a predictive model of human bioavailability.

The pig in the assessment of bioenabling approaches

In formulation development it is crucial to assess the bioavailability of orally delivered drugs, and in particular how this can be influenced by the use of different formulations in order to support the development work. These pre-clinical *in vivo* studies are often used in combination with *in vitro* and *in silico* approaches to gain insights into the biopharmaceutical processes underpinning formulation performance prior to clinical studies. Optimizing the predictive capacity from *in vitro* analysis, through *in vivo* performance in preclinical animal studies, incorporating *in silico* tools, can therefore help to expedite the development process. An important consideration in this approach is the ability of the chosen animal model to discriminate between formulations based on *in vivo* performance.

In this regard, the pig has been widely used in the assessment of bioenabling approaches in early drug development, particularly with fenofibrate as a model compound. The bioenabling approaches used in these settings have included lipid-based formulations (Griffin et al., 2014, Thomas et al., 2014), mesoporous silica and nanosized formulations (McCarthy et al., 2017), and micronised formulations (Thomas et al., 2014) and has proven effective in discriminating between formulation performance.

The current thesis has investigated three novel fenofibrate containing bioenabling formulations; a mesoporous silica based suspension with a precipitation inhibitor (chapter two: FF-SLC: HPMCAS (4:1) suspension), a mesoporous silica based capsule formulation with a precipitation inhibitor (chapter two: FF-SLC: HPMCAS (4:1) capsule) and a spray dried lipid based formulation (chapter three; lipidic dispersion),

along with a commercial micronised formulation (chapter three; Lipantil® Micro) and a crude reference formulation. The observed bioavailability, along with the dose normalised C_{max}, for these formulations as well as those of previously investigated formulations from similar studies in published literature (Griffin et al., 2014, McCarthy et al., 2017) are compared in figure 6-3 in order to assess the ability of the pig model to discriminate between formulation performance. The figure has been divided based on the formulation platform utilised where crude reference is shaded black, commercial preparations based on size reduction (micronisation and nanonisation) are shaded grey, lipid based formulations are shaded blue and mesoporous silica formulations are shaded red.

As presented in figure 6-3, all the investigated formulations demonstrate improved bioavailability relative to the reference fenofibrate (19.92%). While a relatively broad range of increases in bioavailability were observed across the range of formulations (54.55% for SBA-15 – 86.69% for FF-SLC: HPMCAS (4:1) capsule), there is no clear distinguishing feature between the formulations. Two commercial preparations are included in the analysis, the micronised Lipantil® Micro capsule formulation and the nanocrystal formulation Lipantil® Supra. Bioavailability from the Lipantil® Supra capsule in pigs (71%) is comparable to that observed in humans (69%) (McCarthy et al., 2017, Zhu et al., 2010), however absorption from the micronised capsule appears to be higher than anticipated. While no studies of absolute bioavailability for micronised capsule formulations have been conducted in humans, an increase in bioavailability of approximately 30% - 50% can be expected in nanosized relative to micronised formulations (Guichard and Sauron, 1993, Guivarc'h et al., 2004). Overall, while there are obvious increases in fenofibrate bioavailability, it has proven difficult

209

to discriminate between performances of the different types of enabling formulation. While the pig has shown ability in demonstrating proof of concept for enabling formulations, it remains to be determined if a quantitative assessment can be achieved. One clear trend which has emerged, however, is the relative increase in rate of absorption from lipid solutions relative to solid dosage forms, demonstrated here by the relatively higher C_{max} observed for lipid solutions (e.g. Type IIIA & IV LBFs) compares to the other formulations. This is likely due to the fact that the drug is presented in a pre-solubilised form, therefore avoiding the drug dissolution step that is necessary when a drug is presents in a solid crystalline form.
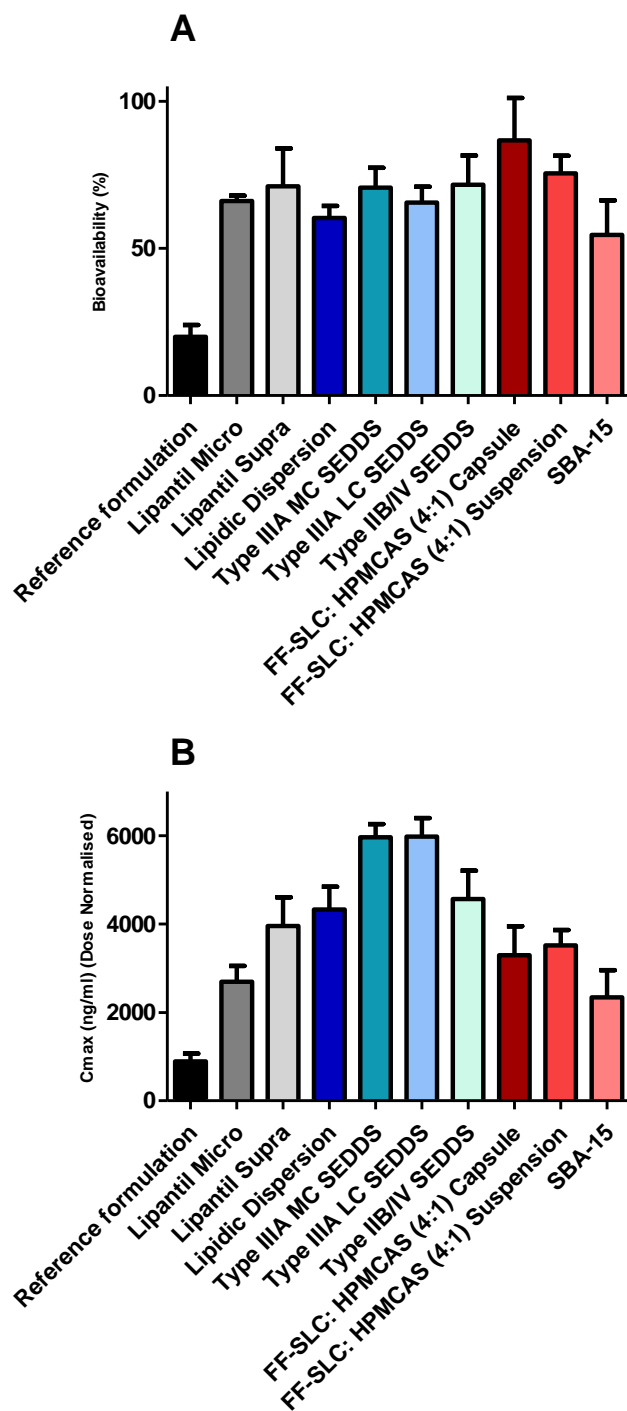
Figure 6-3 Summary pharmacokinetics of fenofibric acid following oral delivery of fenofibrate from:

- *Reference formulation (67mg, n = 6) – chapter two*
- *Lipantil® Micro (67mg, n = 4) – chapter three*
- *Lipantil® Supra (67mg, n = 4) - (McCarthy et al., 2017)*
- *Lipidic dispersion (67mg, n = 4) – chapter three*
- *Type IIIA MC SEDDS (96mg, n=5) - (Griffin et al., 2014)*

- *Type IIIA LC SEDDS (96mg, n=6) - (Griffin et al., 2014)*
- *Type IIIB/IV SEDDS (96mg, n=5) - (Griffin et al., 2014)*
- *FF-SLC: HPMCAS (4:1) capsules (67mg, n =6) – chapter two*
- *FF-SLC: HPMCAS (4:1) suspension (67mg, n=6) – chapter two*
- *SBA-15 (67mg, n = 4) - (McCarthy et al., 2017)*

(a) Displays Bioavailability (mean ± SD), (b) displays Dose normalised C_{max} (mean ± SD).

The pig as a model to assess food effect

Along with the ability to screen novel formulations, the bioenhancing capability of administering fenofibrate with food in pigs was also assessed. One of the aims of the current thesis was to design a formulation which could over-come food mediated alterations in bioavailability (chapter three) and subsequently assess its ability *in vivo*. As part of this process an effective food effect model in pigs was required, and the design and implementation of such a model was the primary aim of chapter five. The design of the porcine food effect model was ultimately based on a previously published dog model (Lentz et al., 2007), taking into consideration a previously unsuccessful attempt to adapt this model to pigs (Christiansen et al., 2015). Despite consideration to the limitations of previous studies, both with regard to the content of the meal utilised (Christiansen et al., 2015, Henze et al., 2018a) and to the optimal fasting period prior to study commencement (Suenderhauf et al., 2014), it was not possible to induce an increase in fenofibrate bioavailability in the fed state. Thus, while the lipidic dispersion formulation described in chapter three is postulated to eliminate food effect, we have not been successful, thus far, in generating a porcine food effect model to further investigate this hypothesis. This point is further discussed, with possible explanations and solutions suggested, in the proceeding section when assessing the accuracy of *in silico* models of porcine bioavailability.

One area of particular concern when in pig models of bioavailability is that of variable gastric emptying. Previous experience has demonstrated that insufficient fasting periods prior to absorption studies can lead to incomplete emptying of gastric contents, meaning a true 'fasted' state may not be obtained. This is a potential

explanation for the differences in bioavailability for Lipantil® Micro in the fasted state, as observed in chapter three (66.1% ± 3.5%) and chapter five (38.8% ±8.5%) and as presented graphically in figure 6-4. While some of this variation can be attributed to the inherent variability of *in vivo* studies of absorption, it also may reflect the observation that the previous fasting regimen was insufficient to ensure complete emptying of stomach contents. Hence pigs were not dosed in a 'truly' fasted state. The fasting protocol implemented in chapter five, consisting of a small (175g; circa 10.5g/kg) meal 24 hours prior to dosing (24 hour b.d.), ensured adequate fasting, with no significant residual contents observed in the GIT post-mortem. However, the previously implemented feeding regimen in chapter three, consisting of a larger (500g meal) 24 hours prior (24 hour o.d.) to dosing, did not successfully lead to complete gastric emptying, with residual gastric contents observed post-mortem, indicating these animals are not in a truly fasted state. The retention of significant quantities of food in the GIT, even after a 24 hour fast, is a possible explanation for the increased bioavailability seen in the earlier study, suggesting that to ensure bioavailability studies in pigs are truly reflective of the fasted state, the updated fasting protocol should be implemented. This data also suggests that it is indeed possible to demonstrate a food related increase in bioavailability in pigs, however further studies are required for model characterisation and refinement.
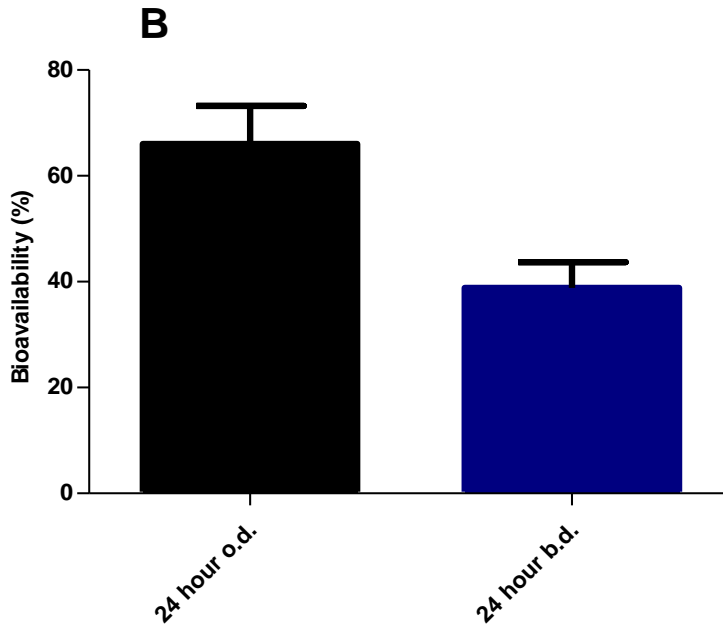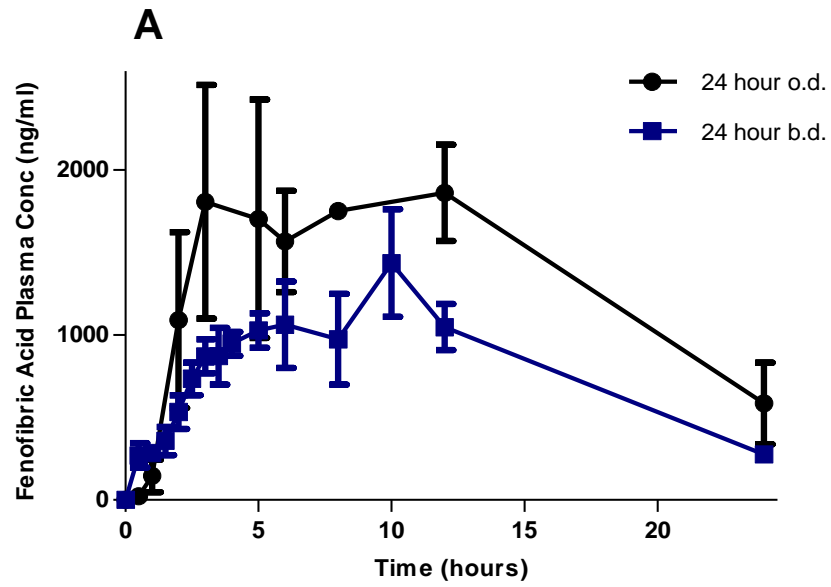
Figure 6-4 (A) Plasma concentration of fenofibric acid vs. time profiles after oral administration of 67mg to fasted pig, all data mean ± SE, 24 hour o.d. (n=4), 24 hour b.d. (n=3)

(B) Fenofibrate bioavailability in fasted pigs, all data mean ± SE, fasting regimen A (n=4), fasting regimen B (n=3)

The predictivity of porcine *in silico* models of bioavailability

One of the aims of the current thesis was to streamline the formulation process by means of assessing the predictive capacity of early *in vitro* screening and identifying the relationship between *in vitro* screening tools and *in vivo* performance in pigs, through the use of *in silico* approaches including IVIVC and PBPK modelling. A key consideration when assessing *in vitro- in vivo* relationships is the biorelevance of the *in vitro* screening method being utilised (Kostewicz et al., 2014a, Kostewicz et al., 2014b). In this regard, chapter three addressed the use of biorelevant dissolution media, demonstrating its superiority over compendial or FDA recommended media in discriminating between performances of varying formulations. Thus, utilisation of such biorelevant dissolution tests is an appropriate starting point for *in vitro* assessment for bioenabling formulations, particularly with regard to elucidating *in vitro-in vivo* relationships.

The simplest methods of predicting *in vivo* results using *in vitro* input is by means of an *in vitro- in vivo* correlation (IVIVC), which directly links *in vitro* and *in vivo* parameters, and numerous groups have demonstrated such relationships in the pig model. Kesisoglou *et al.* demonstrated the use of a simple level C IVIVC in a retrospective analysis of the *in vivo* performance of extended release matrix and multi-particulate preparations of a BCS class III development candidate (MK-0941) with different targeted release rates (8hr, 12hr and 16 hr) in Yucatan minipigs. A good correlation between the *in vitro* release and bioavailability was reported (Kesisoglou et al., 2014). Similarly, Keohane *et al.* demonstrated an *in vitro- in vivo* relationship for coated microspheres containing Ciclosporin A. The resultant IVIVR demonstrated

215

a strong linear correlation between *in vitro* release and *in vivo* absorption (Keohane et al., 2016). McCarthy *et al*., meanwhile, demonstrated the possibility of obtaining a level A IVIVC using such an approach, by optimising the biorelevance of the dissolution test for fenofibrate, as a model poorly water soluble compound. McCarthy and co-workers demonstrated that computational *in silico* methods could be used to deconvolute the oral absorption process from the pharmacokinetic profile, correlate this with *in vitro* release and model *in vivo* pharmacokinetics by re-convolution (McCarthy et al., 2017). Govender *et al.* have recently proposed a similar approach to describe the absorption of amoxicillin from a delayed release, dual-biotic system (Govender et al., 2016).

In chapter two, the relationship of *in vivo* performance to simple *in vitro* screening tools has been further explored. By utilising a straightforward dissolution test, with optimised biorelevance through the use of media simulating the composition of the intraluminal intestinal fluid it was possible to consolidate both the *in vitro* and *in vivo* findings reported in the study, demonstrating an IVIVR between dissolution efficiency and bioavailability. This implies that increases in *in vitro* dissolution of fenofibrate from silica formulations are translated into enhancement of *in vivo* dissolution and therefore oral bioavailability. However, a couple of limitations remain with the current approach, most notably the use of simulated intestinal fluid which mimics human intestinal conditions (FaSSIF-V2) and the low number of different formulations utilised (three) limits the applicability of the model and its quantitative potential.

The potential for utilisation of simple linear relationships between *in vitro* and *in vivo* performance is relatively limited for immediate release, bioenabling formulations, where factors other than those assessed *in vitro* (dissolution in this case), including absorption and gastrointestinal transit, play a role in controlling the rate and extent of bioavailability. In these scenarios, one the most useful ways of assessing the implication of altered *in vitro* performance is through the integration of *in vitro* data with computational *in silico* PBPK models (Kostewicz et al., 2014b, Kostewicz et al., 2014a). The distinguishing feature of PBPK models, relative to empirical computational models, is the application of prior physiological knowledge in the mechanistic mapping of model compartments and in the processes that determine absorption (Suenderhauf and Parrott, 2013, Dressman et al., 2011).

The growing use of the pig as a preclinical species of choice has led to significant developments in porcine PBPK models. The most significant development in PBPK modelling in pigs was the minipig PBPK model developed using the advanced compartmental absorption (ACAT) model and which was subsequently incorporated into the GastroPlus™ simulation program (Suenderhauf and Parrott, 2013). By using a series of mass-balance equations that describe the specific physiology of the minipig, a porcine PBPK model was generated to simulate oral PK. The proposed model was initially validated with griseofulvin and moxifloxacin, with encouraging results (Suenderhauf and Parrott, 2013). However, the authors also identified areas where this model can further be refined, particularly in the areas of absorption related parameters and bile salt profiles within the minipig intestine. Further physiological characterisation along with pharmacokinetic analysis of well-chosen

reference compounds, and adjustment of the model to reflect *in vivo* PK, was suggested to contribute to model refinement (Suenderhauf and Parrott, 2013). Subsequent work using paracetamol as a marker of GI motility and gastric emptying, was used to update the model, which was subsequently validated on a number of PK studies in minipigs using omeprazole, caffeine, midazolam and warfarin. The prolonged gastric emptying in the re-parameterised PBPK model accurately predicted pharmacokinetics of this validation dataset in minipigs (Suenderhauf et al., 2014).

In chapter three, this GastroPlus™ minipig ACAT PBPK model has been used to simulate bioavailability of fenofibrate from a commercial micronized formulation and novel lipid based formulation in fasted landrace pigs. The model successfully simulated fasted bioavailability for both formulations by incorporating the intravenous pharmacokinetic data, along with biorelevant *in vitro* solubility and dissolution measures into the mechanistic model. The model was subsequently used to extrapolate this data to the fed state, where the elimination of a food dependent increase in fenofibrate bioavailability utilising the novel formulation was predicted.

The generation of food effect data in chapter five allowed the opportunity to assess the ability of the previously published predictive PBPK model (from chapter three) of fenofibrate bioavailability from Lipantil® Micro in the fasted and fed state. The predicted fed and fasted state profiles, as well as previously reported data for fasted state administration of Lipantil® Micro from chapter three, and fed and fasted data from chapter five is reproduced in figure 6-5. The pharmacokinetic data from these predicted and observed profiles are summarised in figure 6-6. As can be seen from

figure 6-5A and 6-6A and 6-6B, the PBPK model seems to predict reasonably well the extent of bioavailability in the fasted state, however the rate of absorption is not accurately predicted, with a substantially longer T_{max} observed compared to predicted profile. The most likely cause for this observation is the longer gastric transit observed in the fasted pigs *in vivo* relative to that used in the PBPK model. The standard GastroPlus pig physiology implements a gastric emptying rate similar to human at 0.5 hours. Reducing the rate of gastric emptying will prolong T_{max} and will also likely reduce C_{max} slightly, improving the correlation observed in the current study. A similar finding was observed in the study of Suenderhauf *et al.* (2014), where the initial estimates of gastric emptying in a similar, previously published PBPK model were shorter than that observed by means of paracetamol pharmacokinetics. In their study, Suenderhauf *et al.* re-parameterised this PBPK model using their deconvoluted lag time and gastric emptying rate, before validating the new model with a dataset of four compounds (Suenderhauf et al., 2014). In light of the current work, future PBPK modelling carried out using landrace pigs as a model species should consider the effects of long and variable gastric residence when assessing model fit and performance.

With regard to the fed state data, the predicted profile substantially over-estimates the extent of absorption compared to the observed plasma profile. This further supports the hypothesis that the pig model, as it is currently utilised, is not predictive of human food effect. It appears that FDA breakfast fed pigs do not demonstrate solubility and dissolution enhancing effects that promote absorption of fenofibrate (figure 6-5C and 6-5D). Further characterisation is required to assess the physiological

and physicochemical environment in the fed state pig and in particular the effects of different meal composition on the post-prandial pig gastrointestinal environment. Specifically, the effect of varying quantities of an FDA high fat breakfast on the gastrointestinal environment will provide an insight into the lack of a food effect observed both here and previously (Christiansen et al., 2015). It seems plausible, based on the lack of an observed food effect on the extent of fenofibrate absorption and on gastric emptying, that the current meal caloric content and volume is too small to induce a response.
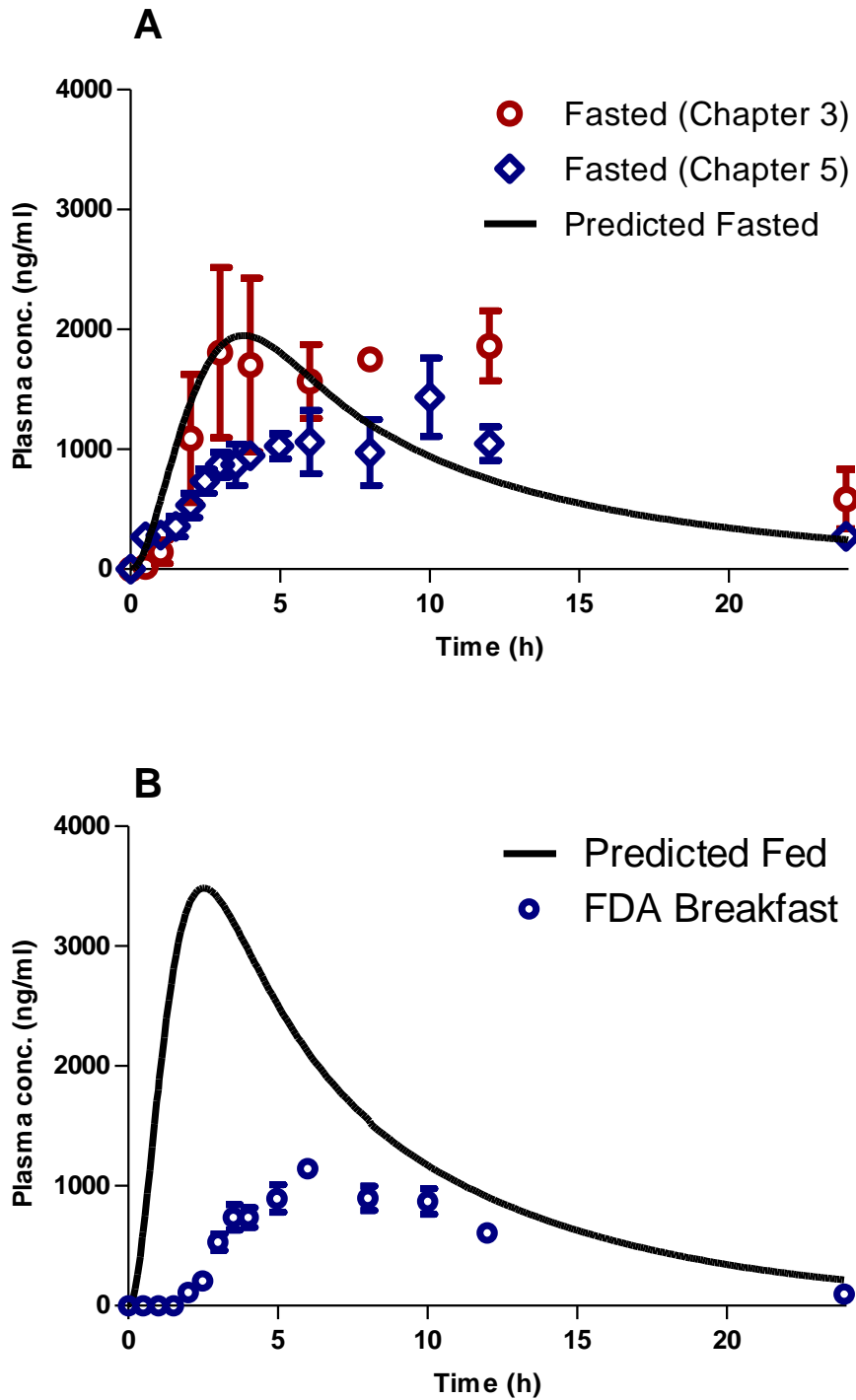
Figure 6-5 Gastroplus™ in silico model of plasma fenofibric acid concentration vs. time profile compared to observed in vivo pig data in the fasted state (A) and fed state (B)

Gastroplus™ Model predicted profiles and fasted data (red) (n = 4, mean ± SE) reproduced from chapter 3

Fasted state data from chapter 5 (blue) (n=3, mean ± range)

Fed state data from chapter 5 (n = 2, mean ± range)
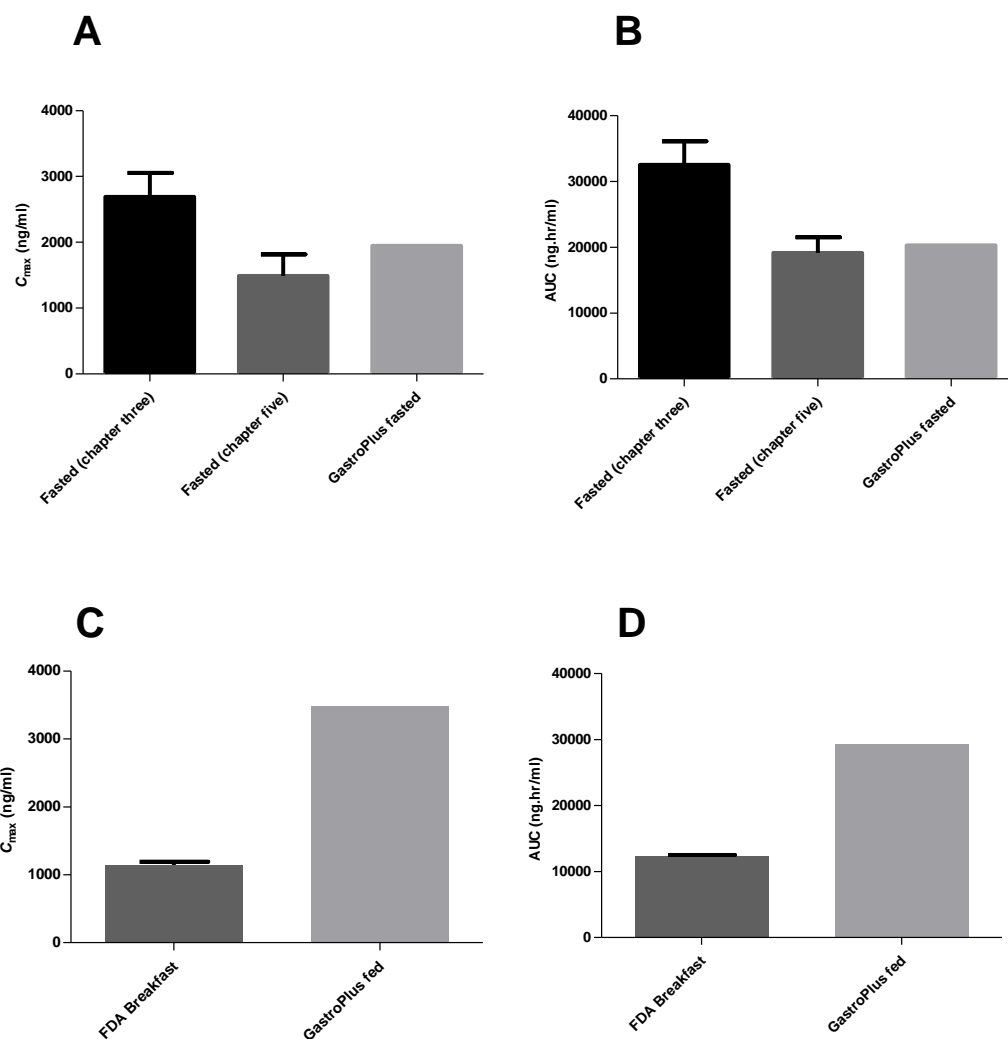
Figure 6-6 Observed vs GastroPlus predicted fenofibric acid pharmacokinetic parameters for Lipantil® Micro in landrace pigs; (A) Fasted C_{max}, (B) Fasted AUC, (C) Fed C_{max}, (D) Fed AUC; fasted state data (black) and GastroPlus model data reproduced from chapter 3

The PBPK modelling approach has since also been utilised elsewhere, with Kesisoglou *et al.* using the GastroPlus™ minipig model, in conjunction with modelling of dog and human data, in the formulation development of a modified release preparation of gaboxadol (Kesisoglou et al., 2015). The authors successfully incorporated *in vitro* dissolution data and preclinical pharmacokinetic data within the PBPK models to

guide formulation development. Subsequently, it was possible to use this minipig PBPK model to develop an IVIVC in order to project formulation performance (Kesisoglou et al., 2015). Using regional permeability data measured in dogs and clinical pharmacokinetics from human studies, the minipig ACAT model was optimised using an immediate release dry filled capsule as a reference. Using this optimised model, the *in vivo* dissolution was deconvoluted from the simulated plasma profile for two modified release formulations. This *in vivo* dissolution profile was subsequently plotted against the *in vitro* release profile resulting in a linear IVIVC (Kesisoglou et al., 2015).

The use of PBPK modelling during drug and formulation development is an emerging field for the prediction of preclinical and clinical PK using physiochemical and *in vitro* measurements. However, thus far the use of PBPK modelling has been largely confined to the retrospective, mechanistic analysis of preclinical data. While some work has focused on the extrapolation of these models to alternative formulations or dosing scenarios (e.g. in fed versus fasted state), and extrapolating between different preclinical species, there is still a lack of prospective models used in formulation design. There remains a need for systematic studies utilising PBPK models as part of a 'learn and confirm' paradigm before the full benefit of this approach is realised.

Overall conclusion and future perspectives

This thesis has, firstly, demonstrated the ability of two novel bioenabling approaches; fenofibrate loaded mesoporous silica in combination with HPMCAS, and a lipidic dispersion to improve oral drug bioavailability. To build on this work, further studies are now needed to address the feasibility of delivering such formulations in human subjects, particularly considering recent success of the first human proof of concept study involving oral delivery of mesoporous silica (Bukara et al., 2016b). In order to elucidate fully the utility of these promising approaches, clinical validation will be required.

Secondly, the utility of *in vitro* and *in silico* tools to predict *in vivo* performance in fasted pigs has been investigated. In particular, the utility of biorelevant dissolution testing to provide both a simple, qualitative indicator of *in vivo* performance through the use of dissolution efficiency calculation, and the integration of biorelevant dissolution and solubility data into a quantitative PBPK model have been investigated. While the PBPK model developed was useful in predicting and modelling bioavailability in the fasted state, extrapolation to the fed state proved difficult. Building on this approach will require both validation of PBPK models with a wider array of compounds, representing different biopharmaceutical classes, as well as further characterisation of the gastrointestinal physiology of pigs, particularly with regard to physicochemical composition of luminal contents, gastrointestinal transit and the absorptive processes, and successful incorporation of these measurements into PBPK models.

Thirdly, the understanding of the pig as a model for both the *in vivo* performance of solid oral dosage forms and the pre-clinical performance of bioenabling approaches has been examined. The ability of the pig to act as a model of human bioavailability for both fenofibrate and celecoxib, as well as its ability to act as an *in vivo* screening tool for bioenabling approaches for poorly water soluble drugs has been assessed. In particular, the need for prudent consideration of the model compounds to be assessed in the pig model, principally with regard to the effect of metabolising enzymes and the need for further characterisation of the metabolic pathways involved in first pass metabolism in pigs has been identified.

Finally, limitations of the pig model for assessment of food effect using current approaches have been identified, and alterations to be made for future characterisation, particularly with regard to meal volume and caloric content, have been made. In order to elucidate the physiological and biopharmaceutical aspects determining drug absorption in fed state pigs, future work should also focus on characterisation of both the gastrointestinal fluid in the fed and fasted state, as well as assessing the effects of future fed state protocols on gastrointestinal transit. Concomitantly, the issue of effective fasting protocols for bioavailability studies in pigs has also been addressed. By demonstrating that the presence of food leads to unpredictable responses in drug absorption in pigs, the criticality of appropriate fasting regimens in pigs has been shown, and regimen which will ensure pigs are in a true fasted state has been proposed.

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227

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243

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