University of Strathclyde Glasgow

Evaluating the Fed State Impact on Drug Solubility and Absorption: A Biorelevant In Vitro Approach

Maria Inês Silva

Strathclyde Institute of Pharmacy and Biomedical Sciences

University of Strathclyde

Glasgow, UK

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Declaration of authenticity and Author's Rights

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

Date: 20th of December 2024

Signed: 

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I am a firm believer in the saying, 'If you want to go fast, go alone; but if you want to go far, go together.' This sentiment deeply resonates with me as I write these acknowledgements. Over the past four years, I dedicated all my time and effort to producing the best work I could manage, culminating in this thesis. However, this journey would not have been the same without the support, encouragement, and contributions of so many people along the way.

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" Started making it. Had a breakdown. Bon appetit."

James Acaster, The Great British Bake Off

Abstract

Biopharmaceutics, which studies the relationship between a drug's physical, chemical, biological, and pharmacological properties and its dosage form, is essential for studying and optimising oral formulations. This thesis explores solubility and dissolution behaviours of drugs using fed and fasted state biorelevant media systems with nine media recipes each, derived from a multidimensional analysis of human intestinal fluids.

The central hypothesis of this thesis is that applying biorelevant media to biopharmaceutical testing can provide improved insights on drug absorption. To test this hypothesis, **Chapter 2** describes the preparation of fasted and fed biorelevant simulated media, as well as the methods to apply these media to measure drug solubility, dissolution and supersaturation. **Chapter 3** compares two approaches for measuring drug solubility in fed state media: multidimensional analysis and Design of Experiments. **Chapter 4** then applies fed state solubility data to a novel Developability Classification System grid, examining population-level variability in solubility. In **Chapter 5** solubility behaviour is analysed to identify patterns that reduce the number of media measurements needed for fed state solubility profiling. **Chapter 6** explores the *in vitro*/in vivo correlation between fasted and fed biorelevant media systems solubility data and published fasted and fed human intestinal solubility values, establishing the potential for the biorelevant system as a bioequivalent model. **Chapter 7** introduces a tool for predicting food effects on drug absorption using solubility measurements and Solubility Limited Absorbable Dose calculations. Finally, **Chapter 8** investigates the impact of media changes on intrinsic dissolution rate and supersaturation, providing new insights into how biorelevant media conditions affect dissolution and supersaturation behaviours in both fasted and fed states.

This thesis makes significant contributions for biopharmaceutics by expanding the limited data available on drug behaviour in the fed state and it uncovers variations in drug solubility and dissolution that single media methods fail to reveal, offering deeper insights into drug performance in more realistic gastrointestinal conditions.

List of figures

List of tables

List of abbreviations

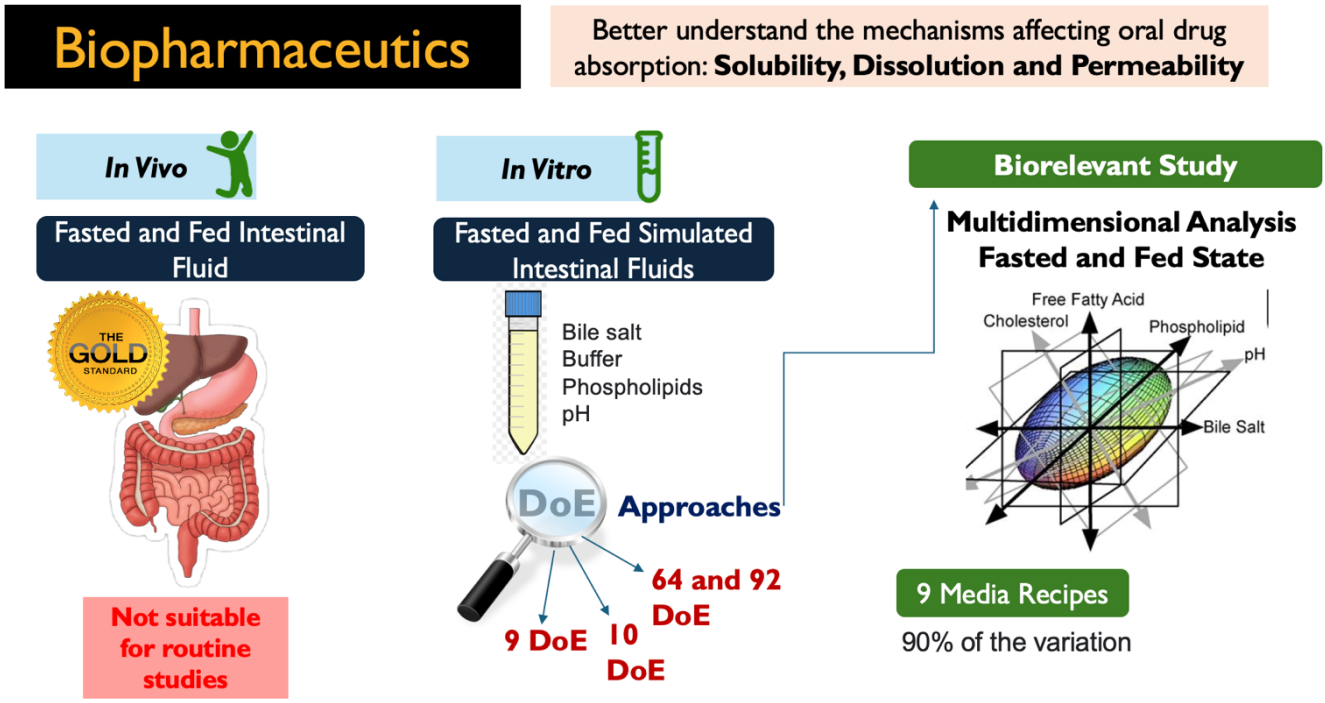
ADME	Absorption, distribution, metabolism, elimination
ACN	Acetonitrile
API	Active Pharmaceutical Ingredients
AUC	Area under the curve
BCS	Biopharmaceutics Classification System
CLint	Intrinsic Clearance (hepatic)
Cmax	Maximum Concentration
CMC	Critical Micelle Concentration
CS	Supersaturation Concentration
DCS	Developability Classification System
DDIs	Drug-drug interactions
DoE	Design of Experiment
DS	Degree of supersaturation
EMA	European Medicines Agency
Fa9SIF	Nine Fasted Simulated Intestinal Fluid
FaHIF	Fasted Human Intestinal Fluid
Fa/FeSSIF	Fasted/ Fed biorelevant nine media system
FaSSIF	Fasted Simulated Intestinal Fluid
FaSSIF-v1	Fasted Simulated Intestinal Fluid Version 1
Fe9SIF	Nine Fasted Simulated Intestinal Fluid
FeHIF	Fed Human Intestinal Fluid
FeSSIF	Fed Simulated Intestinal Fluid Version
FeSSIF-v2	Fed Simulated Intestinal Fluid Version 2
FDA	Food and Drug Administration
GI	Gastrointestinal
HCl	Hydrochloric Acid
HIF	Human intestinal fluid
HPLC	High performance liquid chromatography
IDR	Intrinsic dissolution rate

IVIV	*In Vitro* In Vivo
IVIVC	*In Vitro* In Vivo Correlation
MEC	Minimum effective concentration
MeOH	Methanol
NaCl	Sodium Chloride
$NaH_2PO_4.H_2O$	Sodium Phosphate Monobasic Monohydrate
NaOH	Sodium Hydroxide
OrBiTo	Oral Biopharmaceutical Tools
PC	Phosphatidylcholine
P_{eff}	Effective Permeability
PBPK	Physiologically-based pharmacokinetic
PK	Pharmacokinetics
R	Intestinal Radius
r_p	Particle Radius
SLAD	Solubility Limited Absorbable Dose
SIF	Simulated intestinal fluid
TAC	Total Amphiphilic Concentration
TC	Taurocholate
T_{ind}	Induction time
UK	United Kingdom
UV	Ultraviolet

Table of Contents

Chapter 1

Introduction



This section provides an introduction to the key concepts explored throughout the thesis. It introduces biopharmaceutics, covering fundamental aspects of pharmacokinetics, oral drug absorption, and essential biopharmaceutical measurements such as solubility and dissolution. Additionally, it offers an overview of simulated intestinal fluids and the different approaches to their application.

Each experimental chapter will include a specific introduction related to the themes discussed.

1.1. Biopharmaceutics and Pharmacokinetics

1.1.1. What Is Biopharmaceutics?

Biopharmaceutics is a specialised field within drug development that integrates biological and physicochemical sciences to optimise the in vivo performance of medicinal compounds. Unlike traditional pharmaceutics, which focuses mainly on the preparation and dispensing of medicinal products, biopharmaceutics encompasses a diverse range of scientific disciplines including chemistry, physiology, physics, engineering, and cell biology, to better understand how pharmaceutical products and their active ingredients interact with biological systems. By mastering the principles of pharmacokinetics - absorption, distribution, metabolism, elimination- and drug delivery, biopharmaceutical scientists are able to design and optimise drug delivery systems to ensure that the drug reaches its intended site of action for maximum therapeutic benefits [2, 3].

This multidisciplinary approach primarily focuses on the absorption phase, since it is where dosage form design can significantly impact the drug's pharmacokinetic profile. For example, by understanding how a drug's physicochemical properties affect its absorption, scientists can optimise drug formulations to enhance limiting factors such as solubility, dissolution and permeability, to increase the availability at the target site [4, 5]. In contrast, metabolism and elimination are largely determined by the drug molecular structure, rather than the characteristics of the formulation used for its administration and therefore less explored by biopharmaceutical scientists [2]. In conclusion, by focusing on the critical absorption phase and leveraging the principles of pharmacokinetics, biopharmaceutics bridges the gap between drug formulation and therapeutic efficacy.

1.1.2. The Principles of Pharmacokinetics

Pharmacokinetics (PK) is a critical aspect of drug development and therapy. It involves the quantitative analysis of all the processes that influence a drug’s transit through the body

[2]. The four main stages of pharmacokinetics are absorption, distribution, metabolism, and elimination (ADME) [6, 7]. These processes determine the concentration of the drug at various sites over time and directly influence its therapeutic efficacy and safety profile. Each of these processes is governed by the drug's physicochemical and structural properties and by the biological environment it encounters, making biopharmaceutics a fundamental consideration in pharmacokinetics [2, 6]. In addition, concepts such as pharmacokinetic profile, area under the curve (AUC), bioavailability, bioequivalence, and pharmacokinetic modelling are crucial for bridging the gap between biopharmaceutics and pharmacokinetics and will be further explored in this section.

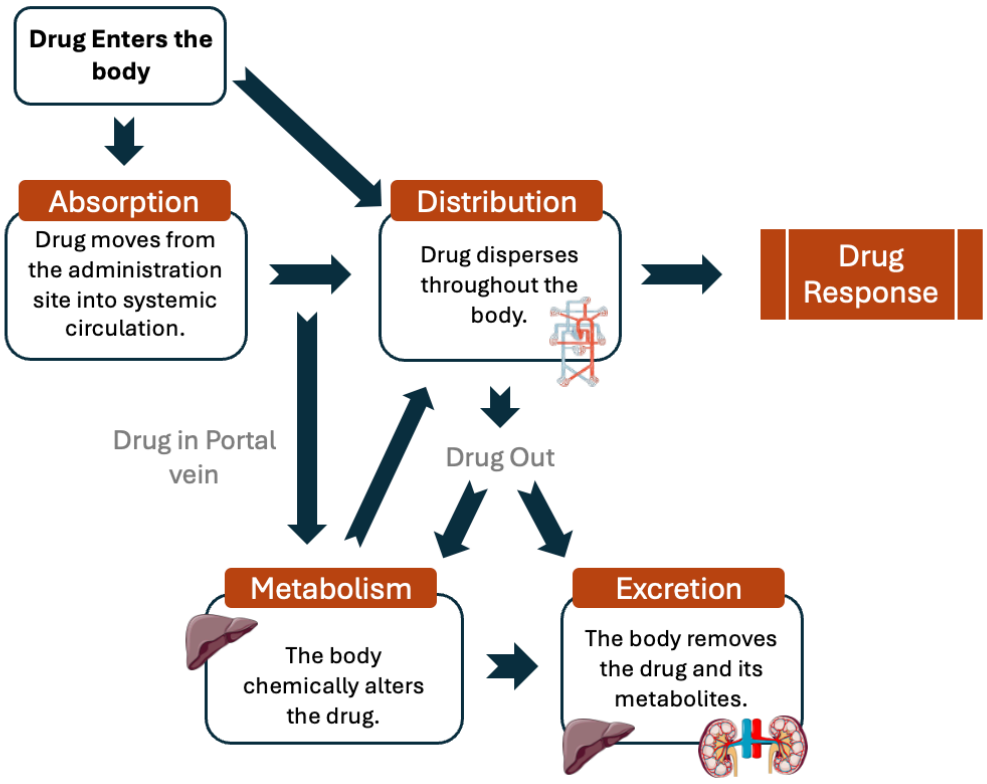


Figure 1. **Illustration displaying the drug cycle within the human body**
Note: Illustration adapted from [6].

1.1.2.1. Absorption

Absorption is the first step in pharmacokinetics, where a drug moves from the site of administration into the blood circulation [8]. The absorption process is heavily influenced by the drug's formulation and the route chosen for its administration which can significantly

impact the pharmacokinetic profile. For instance, drugs administered intravenously bypass the absorption phase and directly enter systemic circulation, leading to immediate bioavailability [9]. In contrast, oral administration requires the drug to pass through the gastrointestinal tract, where it may encounter various barriers and undergo first-pass metabolism before reaching the systemic circulation [10].

This discussion primarily focuses on the oral route of administration, where absorption mainly takes place in the intestinal lumen in a complex process that involves crossing the intestinal membranes into or around the enterocytes. Drugs can be absorbed via [2, 7, 11]:

- Passive Diffusion: Drug molecules move from high to low concentration across cell membranes. This process is influenced by lipophilicity
- Active Transport: Drug molecules are transported against their concentration gradient by specific transporter proteins using energy (e.g., ATP)
- Paracellular Transport: Small, hydrophilic molecules pass through gaps between cells
- Endocytosis: Larger molecules or particles are engulfed by the cell membrane.

The rate of absorption indicates how quickly a drug enters the systemic circulation, while the extent of absorption reflects the total amount of drug that reaches the bloodstream. Together, these factors determine a drug's bioavailability, which is the fraction of the administered dose that reaches the systemic circulation [2]. The absorption process can be influenced by multiple factors such as tablet disintegration, solubility and dissolution, ionisation equilibria, lipophilicity, and interactions with transporter systems [12].

1.1.2.2. Distribution

Distribution is the process by which a drug disperses throughout the body after entering the systemic circulation. The extent of compound distribution and the amount of compound present in the body directly influences its concentration in plasma [9]. This distribution extent is measured by the volume of distribution that represents the hypothetical volume of total body tissues where a drug is distributed following administration. While useful, volume of distribution does not correspond to an actual physiological volume. The true distribution volume of a compound is intrinsically linked to body water and is constrained by the total body water capacity represented by the blood volume (5 L), the interstitial fluids (~11 L) and

the intracellular fluids (~28 L) [6, 9]. A drug with a low volume of distribution typically indicates that it is largely confined to extracellular spaces such as blood plasma and interstitial fluids, rather than widely distributing throughout body tissues [6].

Initially, drugs rapidly distribute to highly perfused organs such as the heart, liver, and kidneys, followed by slower distribution to less-perfused tissues like muscle and fat [6, 13]. Drugs can also bind to plasma proteins serving as a reservoir that maintains the drug's presence in the bloodstream for an extended period.

The extent and rate of distribution are influenced by the drug's physicochemical properties, including lipophilicity, molecular size, and ionisation state, as well as biological factors like blood flow, capillary permeability, and tissue binding affinities [6, 7].

Understanding these dynamics is crucial for optimising therapeutic efficacy and minimising side effects.

1.1.2.3. Metabolism

Metabolism is the process by which the body chemically alters drugs, typically to facilitate their excretion. This biotransformation occurs primarily in the liver through enzyme-mediated reactions. Orally administered drugs must pass through the liver before entering the bloodstream, where they undergo "first-pass metabolism" [6]. This process can reduce the drug's concentration in systemic circulation, as some of it is metabolised before it reaches other parts of the body. Although absorption might be efficient, first-pass metabolism can significantly reduce the drug’s overall systemic exposure [2].

Metabolism can be divided into two phases: Phase I reactions, such as oxidation, reduction, and hydrolysis, often mediated by cytochrome P450 enzymes. Phase II reactions involve conjugation processes, where the drug or its Phase I metabolites are linked with endogenous molecules like glucuronic acid or sulphate, increasing their water solubility to be easily excreted renally [6]. These metabolic transformations can either deactivate the drug or, in some cases, produce active metabolites (that could be desirable in the case of prodrugs) or even toxic metabolites [2]. Understanding drug metabolism is essential for predicting drug interactions, optimising dosing regimens, and ensuring drug safety and efficacy.

1.1.2.4. Elimination

Elimination is the final phase of pharmacokinetics, where the body removes the drug and its metabolites, primarily through renal and hepatic pathways [6]. The kidneys play a major role in excreting drugs into urine, while the liver metabolises drugs for biliary excretion and to facilitate renal excretion. Renal elimination involves glomerular filtration, tubular secretion, and reabsorption processes, determining the drug's half-life and clearance rate [9]. The liver's role includes biotransformation of drugs into more water-soluble forms through glucuronidation and amino acid conjugation facilitating renal excretion [14]. Other elimination routes include faeces, sweat, saliva, and expired air. Effective elimination ensures the drug's therapeutic effects are achieved without accumulation to toxic levels. The elimination half-life of a drug is a critical parameter that influences dosing frequency and duration of action [6]. Understanding the elimination pathways aids in developing strategies to mitigate the effects of renal or hepatic impairment on drug clearance.

1.1.2.5. Pharmacokinetic Profile

A drug's pharmacokinetic profile (Figure 2) represents the concentration of the drug in the blood over time following its administration until most of the drug is eliminated from the body. This profile is typically depicted as the function of plasma concentration versus time (hours), with variations depending on the administration route [6]. Intravenous (IV) drugs have a distinct pharmacokinetic profile: since the drug is directly introduced into the bloodstream, it becomes immediately available in the circulation, reaching maximum concentration quickly and then gradually declining as it is eliminated [6, 15]. In contrast, orally administered drugs have an initial absorption phase where the drug moves from the intestinal lumen into the bloodstream, causing a gradual increase in plasma concentration over time [6,

15]. Once the drug enters the bloodstream, it undergoes metabolism and excretion, leading to a decrease in plasma concentration.

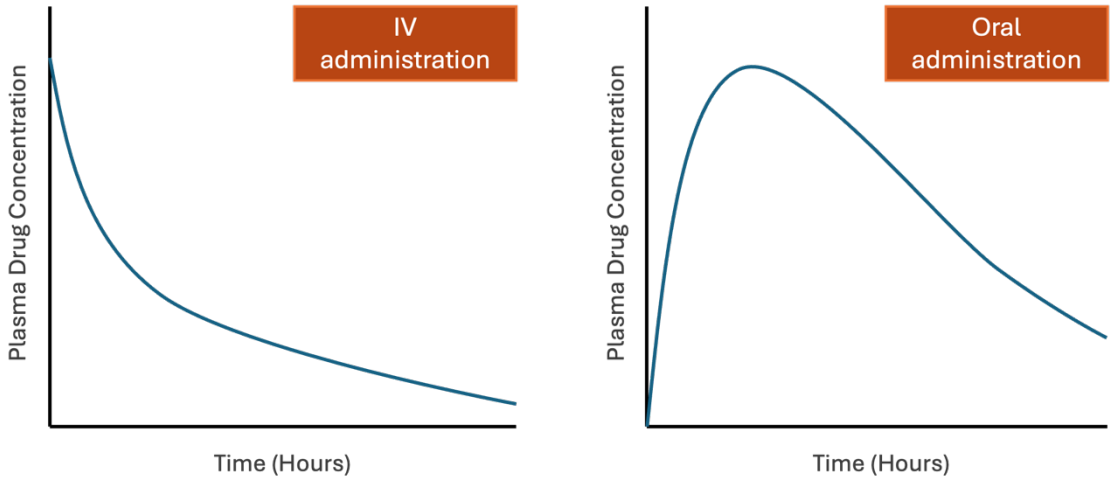


Figure 2. **Pharmacokinetic profile of a drug after intravenous (IV) administration vs oral administration: Typical plasma concentration-time profile**

The AUC (Figure 3) is a key pharmacokinetic term, referring to the total drug concentration in circulation over time, calculated as the area under the plasma concentration-time curve [16]. It is usually expressed as either $AUC_{(0-t)}$ when referring to the total drug concentration from the administration of dosage to the last point of measurement in a pharmacokinetic profile or $AUC_{(0-\infty)}$ when referring to drug concentration from administration to an infinite time [6].

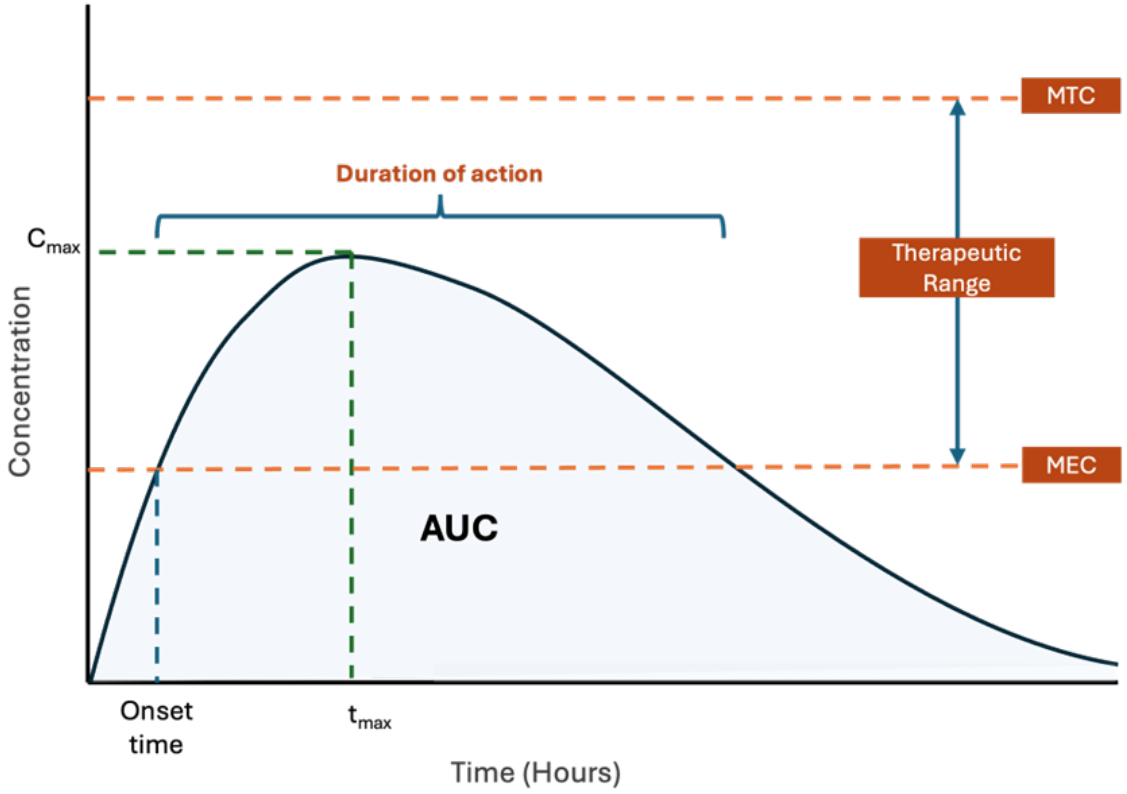


Figure 3. **Pharmacokinetic profile after oral drug administration**

Note: Illustrating the most common pharmacokinetic parameters: Maximum plasma concentration (Cmax), time until Cmax (Tmax), onset time, Minimum effective concentration (MEC), Minimum toxic concentration (MTC) and Area under the curve (AUC). Adapted from [6].

Closely related to AUC is the concept of maximum plasma concentration (C_{max}), which represents the highest plasma concentration in a drug's pharmacokinetic profile [6]. The time it takes to reach C_{max} after drug administration is called T_{max}. The therapeutic effect of a drug is observed when its concentration surpasses the minimum effective concentration (MEC) [6]. The time required to reach the MEC is known as the onset time, and the duration during which the drug concentration remains above the MEC is referred to as the duration of action. A shorter onset time results in a faster therapeutic effect, while a longer duration of action indicates prolonged therapeutic efficacy, both of which are crucial parameters for biopharmaceutical scientists [6]. To determine a drug's safety window, the minimum toxic concentration (MTC) or maximum safe concentration (MSC) is often established. A wider safety window indicates a safer drug. For drugs with a narrow safety window, stricter dosage regimens are necessary to ensure the concentration stays above the MEC without exceeding the MTC, thus preventing toxic effects [6].

Understanding these concepts is essential for optimising drug formulations and ensuring effective and safe therapeutic outcomes. By manipulating these parameters, biopharmaceutical scientists aim to enhance drug performance, reduce adverse effects, and improve patient compliance, ultimately leading to better therapeutic efficacy and safety.

1.1.2.6. Bioavailability

Bioavailability, as previously mentioned, is the fraction of the administered drug dose that reaches the systemic circulation. Ensuring high bioavailability of drugs is therefore essential for achieving optimal therapeutic effects. Regulatory agencies such as Food and Drug Administration (FDA) [17] and The European Medicines Agency (EMA) [18] define bioavailability as a key pharmacokinetic measure used to evaluate the absorption rate and extent of active substances in drug products. It is typically assessed through pharmacokinetic studies comparing the plasma concentration of the drug over time in humans, specifically focusing on parameters like peak concentration (C_{max}) and AUC as markers of absorption.

According to the FDA, bioavailability assessments are essential in new drug applications (NDAs) and investigational new drug applications (INDs) to ensure consistency in therapeutic effects across different formulations or routes of administration. Similarly, the EMA emphasises bioavailability as necessary to establish the therapeutic equivalence of generic products compared to the innovator drugs, ensuring that any differences in formulation do not impact safety or efficacy outcomes.

Bioavailability is heavily influenced by biopharmaceutical factors such as drug solubility, permeability, stability, and formulation design. This concept will be further discussed in Section 1.2.

1.1.2.7. Bioequivalence

Bioequivalence is defined by regulatory agencies as a key requirement to ensure that a generic drug product shows the same rate and extent of absorption as the reference product when administered at the same molar dose under similar conditions. The FDA's regulatory definition of bioequivalence includes strict guidelines to ensure comparable therapeutic performance between the test and reference products and specifies bioequivalence in terms

of pharmacokinetic parameters like the maximum concentration (Cmax) and the curve AUC. For a product to be considered bioequivalent, these parameters must generally fall within an 80-125% range of the reference product's values, measured within a 90% confidence interval. This range is designed to ensure similar therapeutic and safety profiles. The FDA also specifies that bioequivalence comparisons are valid only between products with similar release mechanisms. For example, immediate-release formulations cannot be compared to sustained-release or extended-release versions, as these formulations alter how quickly and how long the drug remains available in the bloodstream. This distinction helps ensure that absorption differences do not alter the drug's therapeutic effect

Biopharmaceutics plays a key role in achieving bioequivalence by ensuring consistent drug release and absorption characteristics across different formulations. This evaluation ensures that patients receive the same therapeutic benefits from generic drugs as they do from their branded counterparts.

1.1.2.8. Pharmacokinetic Modelling

Mathematical models are widely used to analyse and predict PK profiles of drugs after intravenous and oral administration in both animals and humans [19]. These models serve as a powerful tool to predict a drug's behaviour within the body.

Physiologically-based pharmacokinetic (PBPK) modelling incorporates physiological data, such as organ size, blood flow rates, and tissue composition, alongside drug-specific properties, allowing for simulation of drug absorption, distribution, metabolism, and excretion within different tissues [20]. PBPK modelling is routinely applied from early drug discovery stage, where limited data is captured for any compound of interest, to late drug development, where large amounts of data are available [21]. It is a valuable tool for predicting human PK profiles, identifying factors that may limit bioavailability, assessing potential food effects, evaluating the likelihood of significant drug-drug interactions (DDIs) in humans and for determining the *in vitro* properties needed to achieve a target pharmacokinetic-pharmacodynamic (PK-PD) profile [21]. Another great advantage of its application is that it offers the opportunity to study the PK profiles of populations for which clinical trials might not be conducted and enables predictions of drug behaviour across

diverse patient populations, including children, the elderly, and individuals with disease states [22].

As a regulatory agency, FDA [23] has been progressively more accepting of the inclusion of PBPK data in their submission packages, releasing a guidance for the industry on the use of PBPK modelling for certain drug products. Ongoing collaboration between PBPK scientists, industry, and regulatory agencies is essential to clarify core principles of PBPK modelling to enhance regulatory knowledge and build trust in these tools. Strengthening this understanding will ultimately lead to wider acceptance and integration of PBPK modelling in regulatory practices, benefiting both drug development and patient outcomes.

1.1.2.9. Biopharmaceutics Classification System

The Biopharmaceutics Classification System (BCS) emerged in the mid-1990s as a pivotal framework designed to streamline the evaluation of drug absorption in the gastrointestinal (GI) tract. Introduced by Amidon *et al.* (1995)[24], the BCS is grounded in the principle that the absorption of drugs via passive diffusion is primarily influenced by the drug's solubility at the luminal-epithelial interface and its permeability across the intestinal endothelium. This system categorises drugs based on their solubility and permeability, which are critical factors affecting the pharmacokinetic behaviour of oral drug products.

The BCS classifies drugs into four categories: high solubility/high permeability (Class I), low solubility/high permeability (Class II), high solubility/low permeability (Class III), and low solubility/low permeability (Class IV) [24] (See Figure 4).

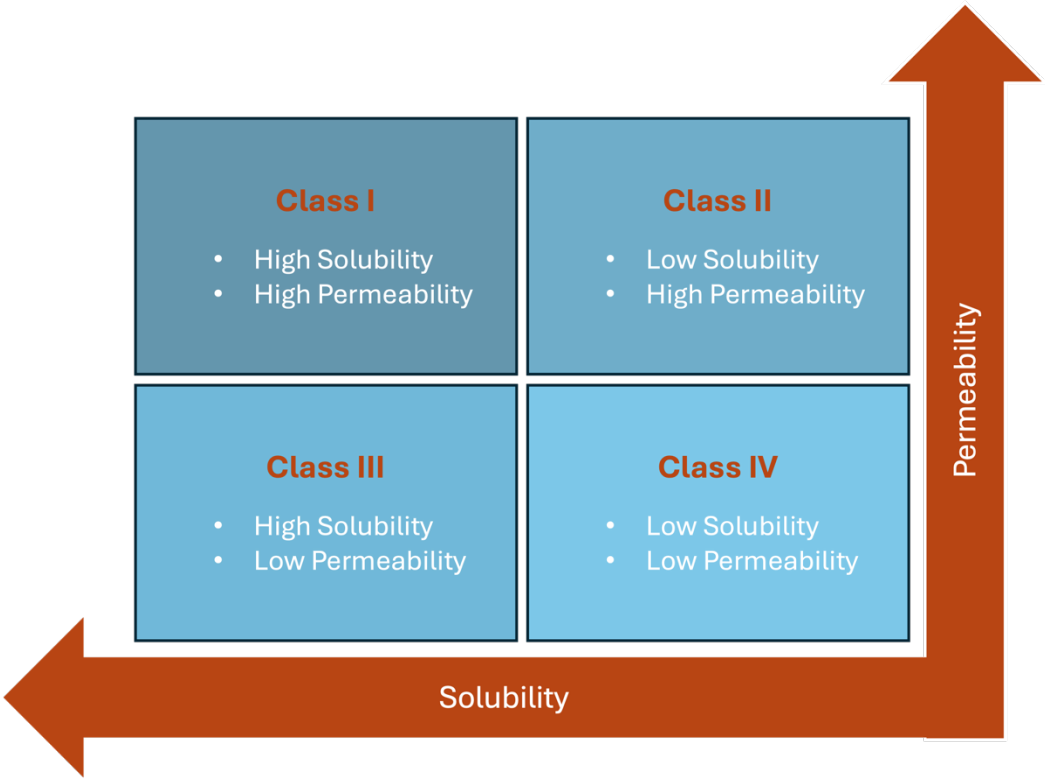


Figure 4. **The Biopharmaceutics Classification System (BCS) and categories**

The solubility criteria within the BCS are based on the assumption that the entire dose is soluble when ingested with a standard glass of water [24]. This approach ensures that solubility is not a rate-limiting factor for absorption. However, there has been ongoing debate about the appropriate media to use for solubility assessments to accurately reflect the conditions in the GI tract without any agreement being reached [25, 26]. In vivo conditions are more dynamic than *in vitro:* drug dissolution creates a concentration gradient that drives absorption, but the extent and rate of permeation across the gastrointestinal membrane depend not only on the drug's solubilisation but also on factors such as membrane permeability, the presence of transport proteins, and local physiological conditions. Thus, dissolution data captures the rate of solubilisation of drugs and is also a crucial part of the BCS criteria [26]. The dissolution criteria requires that the majority of the drug is soluble within the time frame of gastric emptying ensuring that there is no delay in the drug's permeability within the small intestine, thereby facilitating optimal absorption [26]. The permeability criteria within the BCS focus on the extent to which a drug can cross the intestinal membrane and reach systemic circulation [24]. High permeability is typically defined by the absorption of more than 85% of the drug, and various *in vitro* methods, such

as Caco-2 cell assays, can be used to estimate permeability before conducting in vivo studies [24, 26].

The classification of drugs into these categories has significant implications for drug development and regulatory approval processes [26]. For instance, drugs classified as BCS Class I are expected to exhibit rapid and complete absorption, making them less sensitive to formulation variables. Conversely, drugs in BCS Class II or IV may face challenges due to low solubility, requiring advanced formulation strategies to enhance dissolution and absorption. Similarly, Class III drugs may require approaches to improve permeability to achieve desired therapeutic effects.

This classification system provides valuable insights into the potential impact of formulation and physiological changes on the pharmacokinetic performance of drug products. Regulatory agencies, such as the FDA and EMA, employ the BCS to guide the approval process for NDAs and abbreviated new drug applications (ANDAs) [26]. The BCS framework helps determine whether additional bioequivalence studies are necessary or if biowaivers can be granted for new strengths or modified formulations of existing drugs. This standardised approach simplifies the regulatory process and ensures consistent evaluation of drug products [26].

The BCS also serves as a valuable tool for formulators in the pharmaceutical industry. For drugs classified as BCS Class I, novel drug delivery approaches are generally unnecessary, and there is a higher likelihood of achieving formulation equivalence [26]. On the other hand, Class IV drugs pose significant challenges due to limitations in both solubility and permeability [27]. For these drugs, formulation strategies may include the use of solubilising agents to enhance solubility or high-energy solid state forms to improve kinetic solubility [27].

The BCS classification provides a simplified overview of the factors controlling drug absorption [26]. Despite its simplicity, it is often used as a tool during drug development and for guiding formulation strategies.

1.1.2.10. Developability Classification System

The Developability Classification System (DCS) is an extension of the BCS. Created in 2010, the DCS was designed to enhance formulation development by addressing the dynamic and biorelevant aspects of drug absorption that the BCS overlooks [28]. This system is

particularly valuable for identifying critical factors that influence drug absorption and for developing practical formulation strategies [26]. Unlike the BCS, the DCS incorporates biorelevant media for solubility and dissolution assessments. This ensures that testing conditions more closely replicate the physiological environment of the gastrointestinal (GI) tract [28]. Additionally, the DCS employs in silico models and cell-based assays to predict drug permeability, offering a more comprehensive depiction of a drug's absorption profile [28].

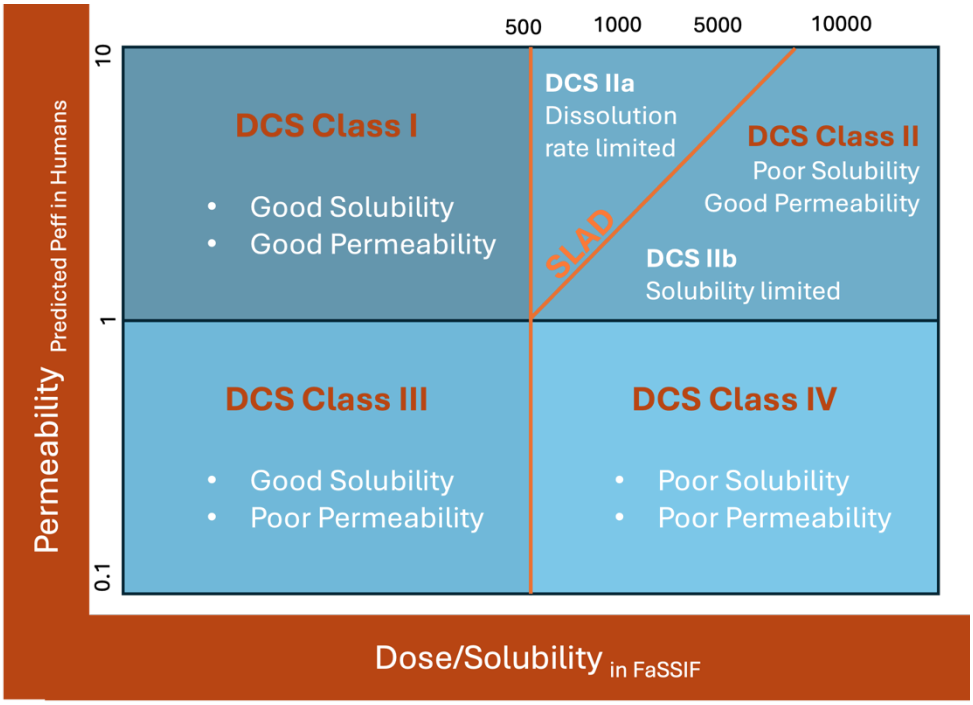


Figure 5. **The Developability Classification System (DCS) and categories**

An important feature of the DCS is its further categorisation of BCS Class II compounds, which have low solubility but high permeability (See Figure 5). These compounds are split into two subgroups based on their rate-limiting absorption step: solubility limited and dissolution rate-limited [28]. This distinction allows for more targeted formulation approaches. For instance, enhancing GI solubility is crucial for solubility limited compounds, while improving the dissolution rate is key for dissolution rate-limited compounds [28].

Understanding the rate-limiting step in drug absorption is central to the DCS, as it guides the development of predictive tests and formulation strategies [26]. For drugs limited by solubility, strategies may include using solubilising agents or developing amorphous solid

dispersions [28]. For those limited by dissolution rate, techniques like particle size reduction or the use of surfactants might be employed [28].

The DCS underwent a significant update in 2018, which incorporated additional considerations to refine the system [29]. The revised version addressed the impact of dose ranges and the solubility and precipitation behaviour of drugs in the gastric environment, particularly focusing on poorly soluble weak bases. These enhancements help to mitigate risks in formulation development for these challenging compounds, leading to more accurate predictions of their *in vivo* performance [29, 30].

It is important to note that the DCS is not intended for regulatory biowaivers. Its primary purpose is to inform formulation development and optimise drug delivery strategies during the preclinical and early clinical stages [26]. By identifying potential absorption challenges early, the DCS enables the development of more effective and reliable drug products.

1.2. Bioavailability and Oral Drug Absorption

Bioavailability is a crucial pharmacokinetic parameter defined as the fraction of the active pharmaceutical ingredient (API) that is absorbed from a drug product and becomes available at systemic circulation [31-33]. For drugs not intended for systemic absorption, bioavailability is assessed by measuring the extent and rate at which the active ingredient becomes available at the action site [11].

Mathematically, bioavailability (F) can be represented as:

$$F = F_a \times F_g \times F_h$$ [34, 35] (Equation 1)

Where:

- F_a is the fraction of the drug that permeated through the intestinal membrane and is therefore absorbed
- F_g is the fraction that escapes gastrointestinal metabolism
- F_h is the fraction escaping hepatic first-pass metabolism.

The absorption process plays a critical role in determining bioavailability, influencing both the extent and rate at which a drug reaches its target site to yield therapeutic effects

[31]. When drugs are administered orally and pass through the gastrointestinal tract, their absorption is often a complex process, shaped by physiological factors of the gastrointestinal tract and physicochemical characteristics of the drugs [11]. Consequently, their oral bioavailability (F) is typically less than 100% (or less than 1)[6] (Figure 6). In contrast, drugs administered directly into the bloodstream, such as via intravenous injection, bypass the gastrointestinal absorption barriers entirely, allowing the full administered dose to enter the systemic circulation and resulting in a bioavailability (F) of 100% (or 1) [6].

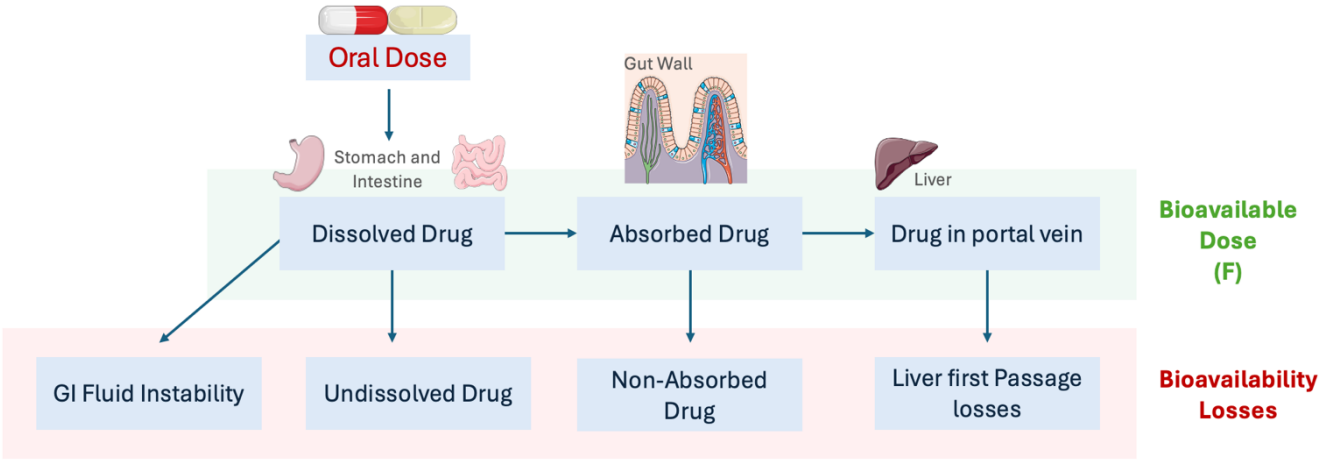


Figure 6. **Illustration portraying the fundamental steps to guarantee bioavailability after oral administration**

To fully understand the impact of drug absorption on bioavailability, it is essential to consider the factors that influence the absorption process. Oral drug absorption predominantly occurs in the small intestine through various mechanisms such as passive transcellular diffusion, carrier-mediated transport, paracellular transport, and endocytosis [11] (See Section 1.1). It is a complex process governed by the interplay between solubility, which is the drug's ability to dissolve in gastrointestinal fluids, permeability, which refers to the drug's capacity to cross biological membranes and enter the systemic circulation, the dosage of the drug and also the surface area of the intestine [11, 24]. These factors, along with others that might affect solubility and permeability [24], play a critical role in determining how much of the drug is absorbed into the systemic circulation and, consequently, its bioavailability. For instance, a drug with excellent solubility but poor permeability may have limited absorption, resulting in low bioavailability. Conversely, a drug with high permeability but low solubility may also exhibit low bioavailability because it cannot

dissolve adequately to be absorbed (See Figure 6). The metabolic stability of a drug also plays a crucial role in its bioavailability. Drugs that are extensively metabolised in the gut wall or liver before reaching systemic circulation (first-pass metabolism) typically have reduced bioavailability [36] (See Figure 6).

Bioavailability will therefore be largely dependent on the factors that affect drug absorption, making it essential to understand these factors for the development of more effective pharmaceutical formulations. The following section will enumerate and expand on some of these key factors, providing a comprehensive understanding of their impact on drug absorption and bioavailability.

1.2.1. Factors affecting Drug Absorption

1.2.1.1. Physiological Factors of the Gastrointestinal Tract

The gastrointestinal (GI) tract is composed of several key anatomical structures, each playing a vital role in digestion and absorption: the mouth, oesophagus, stomach, small intestine, large intestine, anus and accessory organs (liver, pancreas, and gallbladder) [37, 38]. Together, these components work to ensure efficient digestion, absorption, and elimination of both nutrients and medications. Various biological factors within the GI tract, including gastric emptying, pH levels, GI content, motility, surface area, and blood flow, play pivotal roles in drug absorption [39, 40]. Understanding and considering these factors is essential in the design of effective drug delivery systems to improve therapeutic efficacy.

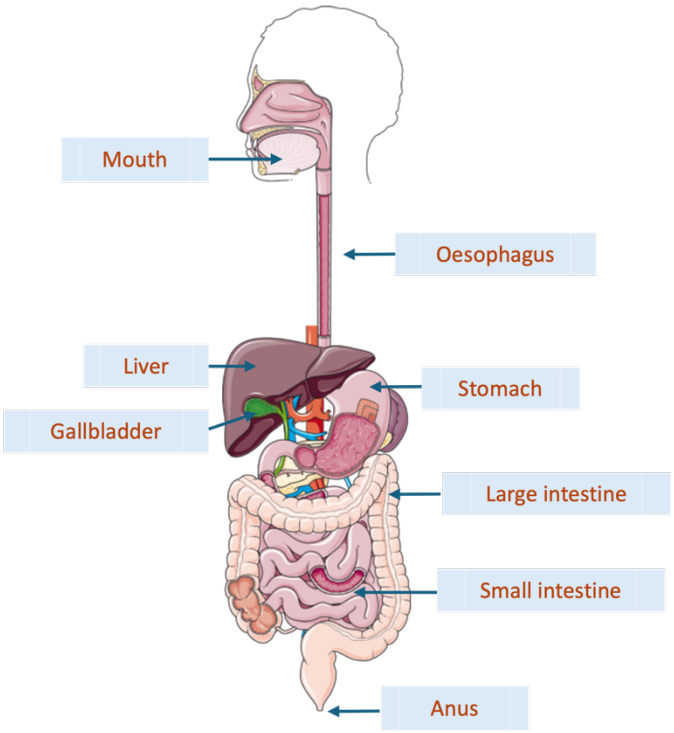


Figure 7. **Illustration representing the gastrointestinal tract and its organs.**

1.2.1.1.1. Gastric Emptying Time

Gastric emptying is the process by which the stomach's contents are moved into the small intestine. The rate of gastric emptying can significantly influence drug absorption because drugs need to reach the small intestine, where most of the absorption takes place [41].

Differences in the gastric emptying times between the fasted and fed state are expected and can also have an impact on absorption, with the fasted state presenting faster rates than in the presence of food [42]. Magnetic resonance imaging shows that the time for the stomach to return to fasted gastric volumes is about 45 minutes after consuming 240 mL of water [43]. However, it takes more than 6 hours after ingesting a high-caloric breakfast, as standardised by the FDA [44].

When gastric emptying occurs more rapidly, it can lead to faster drug absorption. However, if gastric emptying is delayed, the drug remains in the stomach longer, which can increase the risk of degradation by gastric acid and enzymes [40, 41, 45]. For instance, the rate of gastric emptying can negatively affect the efficacy of analgesic drugs like paracetamol

when taken with food, as the drug's prolonged stay in the stomach delays its absorption and therefore its therapeutic effect [46].

1.2.1.1.2. Gastric and Intestinal pH

The pH of the gastrointestinal (GI) tract varies considerably from the highly acidic environment of the stomach, where pH ranges from 1 to 3 in the fasted state [47] and from 4 to 6 in the fed state [48], to the more neutral to alkaline conditions in the intestines, which ranges from pH 6.1 to 7.0 in the fasted state [38] and 5.3 to 6.3 in the fed state [49, 50]. This variation in pH can have a significant impact on drug stability and solubility, which in turn affects drug absorption and will be further explored in Section 1.3.

In the acidic environment of the stomach, certain drugs may experience stability issues. The harsh acidity can cause some drug products to degrade, which decreases the amount of API available for absorption by the time the drug reaches the small intestine [4]. Additionally, the acidic pH can influence the ionisation state of drugs. Many drugs are weak acids or bases, and their solubility is pH-dependent [51]. The influence of pH in drug solubility will be further discussed in Section 1.3.

1.2.1.1.3. Surface Area

The surface area of the GI tract is a critical factor in drug absorption. The small intestine is the major site of drug absorption, due to its large surface area. In adults, the small intestine's surface area is greatly expanded due to the presence of villi and microvilli, which are richly supplied with blood vessels [52]. These small vascular projections called villi, project into the intestinal cavity and move nutrients from the intestinal lumen to the circulatory system [52]. The villi are covered with even smaller projections called microvilli that increase the surface area so that each villi maximises its absorption capacity [52]. This structural adaptation enhances nutrient absorption by providing a vast surface area for interaction with digested substances [52, 53]. A larger surface area means that there is more opportunity for the drugs to come into contact with the absorptive epithelium resulting in greater drug

absorption [53]. Consequently, conditions that reduce the surface area, such as diseases that cause villous atrophy, can significantly impair drug absorption.

1.2.1.1.4. GI Transit Time

GI transit time refers to the period it takes for substances to move through the GI tract. The rate at which a drug passes through different sections of the GI tract determines how long it remains in areas where absorption occurs [11, 54]. Although drug absorption can occur in the stomach, once the drug reaches the small intestine, longer transit times allow more time for the drug to be absorbed, particularly for drugs with slower absorption rates [40]. However, if the transit time is too short, the drug may pass through the absorption sites too quickly, resulting in lower drug absorption and reduced therapeutic efficacy [11]. Some drugs have specific absorption windows where they are absorbed preferentially in particular regions of the intestine, often the proximal small intestine, due to factors such as solubility, stability, and interactions with intestinal enzymes and bile salts [54]. This specificity means that if transit time through these regions is shortened, drug absorption and bioavailability can be limited.

For drugs designed for extended-release or controlled release, the GI transit time becomes critical as they depend on a predictable transit time to release the drug gradually as it moves through the GI tract [11].

The impact of fasted and fed state changes in GI transit time will be further discussed in Section 1.5.

1.2.1.1.5. Intestinal Motility

GI motility refers to the spontaneous movement of the GI tract and it mostly influences the period in which the drugs reside at their absorption sites [11, 55]. Increased motility can reduce the contact time between the drug and the absorptive surfaces of the GI tract and result in decreased absorption [55]. Reduced motility can increase the contact time, potentially enhancing absorption [55]. Drugs that alter GI motility, such as anticholinergics, can thus significantly impact the pharmacokinetics of concurrently administered drugs [55-57].

1.2.1.1.6. Gastrointestinal Fluids

Gastric fluids are composed of several components originating from various sources: substances secreted by the stomach itself (such as gastric acid, enzymes, electrolytes, and mucus), swallowed materials (like saliva, food, and liquids), and materials refluxed from the duodenum, including bile constituents [38]. The enzymes present in gastric fluids play a critical role in the digestion of ingested food, aiding in the initial breakdown of meal components [38].

Intestinal fluids are a complex mixture of secretions originating from various sources along the digestive tract, including bile, pancreatic juice, and fluids secreted by the intestinal mucosa itself [58]. These fluids contain bile salts, cholesterol, phospholipids, enzymes (such as proteases, lipases, and amylases), bicarbonate ions, electrolytes, and mucus, all of which contribute to the breakdown and absorption of nutrients [58, 59]. Enzymes from the pancreas aid in the digestion of proteins, carbohydrates, and fats, while bicarbonate neutralises stomach acid, maintaining an optimal pH environment for enzymatic activity in the intestine [58, 59]. The presence of bile salts, released from the gallbladder into the small intestine, is crucial for the emulsification of fats, enhancing the solubility and absorption of lipophilic drugs and dietary lipids [58, 60](See Section 1.3).

The volume of GI fluids also impacts drug absorption by influencing the concentration gradient between the GI lumen and the absorptive mucosa, impacting the dissolution and solubility of drugs, particularly for poorly soluble compounds [61]. In the fasted state, the stomach fluid volumes range from 25 to 45 mL, the small intestine holds an average fluid volume of around 43 ± 14 mL, and the ascending colon has a fluid volume ranging from 7 to 22.3 mL [43, 62, 63]. In the fed state these volumes can vary depending on the volume of the ingested food, and the time after ingestion [61]. This variability in GI fluid volumes highlights the importance of considering both fasted and fed states in drug formulation and administration to optimise absorption rates and bioavailability. The differences between the two states will be further discussed in Section 1.5.

1.2.1.1.7. Food

The co-administration of drugs with food can greatly affect both the rate and extent of drug absorption, which in turn can alter the drug's therapeutic effectiveness [64]. As stated above, bile salts, fats, and other nutrients can either enhance or inhibit drug absorption [53, 64]. The presence of food can also alter the gastric emptying rate, change the pH of the stomach, and directly interact with the drug, potentially forming complexes that are less readily absorbed [53, 64]. This topic will be explored further in Section 1.5.

1.2.1.1.8. Blood Flow

Blood flow to the GI tract is essential for the transport of absorbed drugs into the systemic circulation. Enhanced blood flow can improve drug absorption by maintaining a concentration gradient that favours drug uptake [11]. On the contrary, reduced blood flow can decrease drug absorption and lead to lower bioavailability [11]. Factors such as exercise, temperature, and disease states can influence GI blood flow and impact drug absorption [8].

1.2.1.1.9. Age

Age can significantly affect drug absorption due to physiological changes that occur throughout life. In older adults, factors such as reduced gastric acid production, slower gastric emptying, and decreased blood flow to the intestines can lead to slower or less efficient drug absorption [65]. In younger populations, such as infants and young children, their immature gastrointestinal systems might contribute to variable absorption rates and different drug bioavailability compared to adults [66]. These age-related differences highlight the need for age-specific considerations in drug dosing and formulation.

1.2.1.2. Molecular and Physicochemical Properties of Drugs

In addition to the factors and characteristics of the GI tract, the molecular and physicochemical properties of drugs—such as molecular size, lipophilicity, ionisation state, particle size, and polymorphism—are also key factors that influence drug absorption [12]. These properties can determine how efficiently a drug is absorbed when taken orally which directly impacts its therapeutic success.

1.2.1.2.1. Lipinski's Rule of 5

Lipinski's Rule of Five is a set of guidelines used to predict the oral bioavailability of a drug based on its molecular properties [67]. The rule suggests that a drug is more likely to be well absorbed if it meets certain criteria:

- A molecular weight of 500 daltons or less
- A log P (lipophilicity) of 5 or less
- No more than 5 hydrogen bond donors
- No more than 10 hydrogen bond acceptors.

While these guidelines offer valuable direction in molecular design and lead optimisation [67], the studies that followed Lipinski's approach and focused on exploring with more detail the factors contributing to low oral bioavailability, have significantly enhanced drug delivery technologies [12].

- **Molecular Weight**

A higher molecular weight leads to a larger molecule, which typically has reduced permeability due to its increased size and volume. Additionally, larger molecules often exhibit poorer solubility because they require more solvent molecules for solvation. For these reasons, the target molecular weight is typically set at less than 500 [68].

- **Lipophilicity**

Lipophilicity is measured by the log P value (the partition coefficient of a drug's solubility in octanol versus water) and plays a crucial role in drug absorption [12, 68]. A drug's log P reflects its balance between hydrophilic (water-soluble) and lipophilic (lipid-soluble) properties [68]. For optimal absorption after oral ingestion, a balance between these properties is necessary: while the aqueous environment of the gastrointestinal tract promotes drug solubility, membrane permeability favours non-ionised, lipophilic compounds [68]. Therefore, a log P value of less than 5 is generally preferred [12, 68]. However, because the pH of the GI tract varies, it is important to consider the partition coefficient in relation to the relevant pH, as this can influence the drug's ionisation and, consequently, its solubility and absorption[68]. Log D, or the distribution coefficient, quantifies a drug's ability to partition between lipid and aqueous phases while accounting for the drug's ionisation state at a specific pH, commonly 7.4. Unlike Log P, which measures partitioning only for the unionised form of the compound, Log D reflects both ionised and unionised forms, making it more relevant for physiological conditions.

- **Number of H-Bond Donors and Acceptors**

A high number of hydrogen bond donors can reduce membrane permeability due to the increased polarity of the compound. Therefore, compounds with fewer than five hydrogen bond donor groups are preferred. Likewise, hydrogen bond acceptors also contribute to polarity, and having fewer than ten acceptors is associated with improved absorption characteristics [68].

1.2.1.2.2. Ionisation and pKa

The ionisation constant, or pKa, is a fundamental property that directly influences a drug's solubility and absorption. Drugs with ionisable groups show pH-dependent solubility, as ionised species are generally more soluble due to increased polarity [2]. Weak acids become more soluble as pH increases, while weak bases show greater solubility at lower pH values[2]. For non-ionisable (neutral) drugs, solubility remains unaffected by pH changes within the gastrointestinal (GI) tract [2]. Since the GI pH varies—from acidic in the stomach

to neutral in the intestine—a drug's pKa determines where along the GI tract it will likely dissolve and be absorbed effectively, impacting its overall bioavailability and therapeutic efficacy [2] (See section 1.3).

1.2.1.2.3. Solid State Properties

The solid state properties of drugs, such as crystal form, polymorphism, and particle size, play a crucial role in influencing drug absorption [12]. These properties affect the drug's solubility, dissolution rate, and ultimately its bioavailability. Understanding and optimising these solid state properties is essential for improving the drug's absorption profile.

- **Polymorphism**

Polymorphism refers to the ability of a compound to exist in multiple crystalline forms, each with different molecular arrangements in the solid state. These polymorphs, along with pseudo-polymorphs (solvates and hydrates), can significantly impact a drug's solubility, crystal shape, and dissolution rate, all of which influence its absorption [12]. Highly soluble drugs are less affected by polymorphism since solubility is not the limiting factor for their absorption however, they can greatly affect the bioavailability of drugs with solubility limited absorption [12]. Therefore, identifying and developing the most thermodynamically stable solid form of a drug is crucial to ensure consistent absorption and preventing formulation challenges [12].

- **Crystal form**

Solids are classified as crystalline or amorphous based on molecular arrangement. Crystalline solids have molecules arranged in a regular, geometric structure, making them stable but less soluble due to the energy needed to disrupt their organised pattern. In contrast, amorphous solids lack this long-range order, leading to higher solubility, which is often preferable for drugs [12]. Thus, the crystal form of a drug significantly impacts its solubility and absorption. Amorphous forms, with their highest free energy, generally offer the greatest potential for increased solubility and bioavailability [12, 69]. Literature indicates

that the solubility of amorphous materials can be improved by factors ranging from less than two-fold to over 100-fold compared to their crystalline counterparts [69].

- **Particle Size**

Particle size plays a critical role in drug absorption because it directly influences the surface area available for dissolution. For drugs whose bioavailability is limited by the dissolution rate, reducing particle size can significantly enhance absorption [70]. Submicron-sized particles, in particular, can further increase absorption; however, these smaller particles tend to agglomerate, which can reduce their efficacy [12]. To counter this, surface-active agents are often added to decrease interfacial tension and stabilise the particles. Various techniques, such as sieving, laser light diffraction, and specialised microscopy, are used to measure particle size distribution, depending on the particle size range being studied [12].

1.3. Solubility

Solubility is a critical measurement in pharmaceutical science, as it directly impacts a drug's bioavailability by influencing its dissolution rate. It can be defined as the analytical composition of a mixture or solution that is saturated with one of its components, typically expressed in terms of concentration or other units [71, 72]. The concept of saturation implies an equilibrium state, which can be stable or metastable meaning that the composition of a system may maintain a particular value for a long time, yet still shift suddenly or gradually to a more stable state if subjected to a specific disturbance [12]. In pharmaceutical literature, these concepts are often referred to as "equilibrium solubility" for stable systems and "kinetic solubility" for metastable systems [67, 72-74].

Historically, poorly soluble compounds were those with solubility in the range of 10–100 µg/mL, with almost no marketed drugs falling below 10 µg/mL [67]. However, advancements in drug discovery such as combinatorial chemistry and high-throughput screening have led to the development of compounds with much lower solubility, often below 1 µg/mL [12]. Inadequate solubility in the gastrointestinal lumen can restrict absorption and

reduce subsequent exposure to the drug, making solubility a critical factor in decision-making and risk assessments during drug development [51].

1.3.1. Aqueous Solubility

Aqueous solubility refers to the ability of a substance to be dissolved in water. It is a measure of how much of a substance (solute) can be dissolved in water (solvent) at a given temperature and pressure, resulting in a homogeneous solution [12]. Aqueous solubility is influenced by two primary factors: heat of solvation and heat of fusion [75]. The heat of solvation, which relates to the energy needed to dissolve a compound in water, is often measured by the octanol/water partition coefficient (log P) [75]. Compounds with low heat of solvation such as lipophilic compounds, are not inclined to interact with water resulting in poor aqueous solubility [12]. For crystalline compounds, an additional factor, the heat of fusion, is necessary to break the crystal lattice and allow the molecule to dissolve [75]. High melting points indicate strong crystal packing interactions, and compounds with such properties generally exhibit poor aqueous solubility unless the heat of fusion is exceeded by the heat of solvation [12].

Although an important measurement in the GI tract, aqueous solubility does not fully reflect a drug's solubility. Factors such as pH variability, the presence of bile acids, and the interactions with other luminal contents can alter how a drug dissolves and behaves in this environment, making aqueous solubility alone an incomplete predictor of a drug's overall solubility and absorption potential within the GI tract [76].

1.3.2. pH and drug Solubility

As previously discussed in Section 1.2.1.1.2**,** the solubility of ionisable drugs, particularly weak acids and bases, is intricately tied to the pH of their environment. Understanding a drug's pKa, which indicates the pH at which the drug exists in equilibrium between its ionised and unionised forms, is key to predicting how pH will affect solubility [12, 77]. Ionised drugs generally have higher solubility in aqueous environments because the charged (ionised) state increases their polarity, allowing for better interactions with water molecules, which are also

polar [77, 78]. The unionised form of a drug generally has better permeability through biological membranes, which is vital for absorption [77, 78]. As the drug equilibrates between its ionised and unionised forms, the unionised portion that permeates the membrane will be replenished, allowing for continued absorption in both the acidic and neutral regions of the gastrointestinal tract [12]. This makes pH solubility profiles an important consideration early in drug development to identify candidates with favourable absorption characteristics across different pH environments.

As the pH shifts throughout the gastrointestinal tract—starting with the acidic conditions in the stomach and moving to the more neutral environment of the small intestine—drug solubility can fluctuate significantly [51]. This fluctuation is especially critical for weakly basic drugs, whose solubility typically decreases in the small intestine, a primary site for drug absorption. Even minor changes in pH can have an impact on the solubility of ionisable drugs, making it essential to conduct solubility measurements in buffered solutions to maintain a stable pH during testing [51].

1.3.3. Solubility As a Limiting Factor to Drug Absorption

Solubility determines how much of a drug can be dissolved in the GI tract and be available for absorption. The minimum solubility required for effective drug absorption varies depending on the drug's permeability, dissolution and dose [51]. In practice, poor solubility often becomes the limiting factor for absorption when the dissolution rate and permeability are sufficient [12]. For instance, even if a drug dissolves quickly and its permeability is high, poor solubility can restrict the total amount absorbed because the gastrointestinal tract may become saturated. When saturation happens, a higher dose does not increase the absolute amount of drug absorbed, meaning that the limiting factor for drug absorption is its solubility [12].

Lipinski's rule [67] suggests that if a drug's solubility is greater than 65 µg/mL, it is generally not expected to be the limiting factor for absorption at a dose of 1 mg/kg, and it predicts that solubility becomes a significant factor if it drops below 10 µg/mL. However, it is difficult to determine exactly when solubility might be limiting since it is often drug dependent

[51]. For instance, drugs with average permeability might present sufficient absorption with lower solubility values but for poorly permeable compounds, a higher solubility might be necessary to ensure sufficient absorption [12].

The link between solubility and absorption is acknowledged by the concept of Maximum Absorbable Dose (MAD). The MAD value is fundamentally the amount of drug with the potential to be absorbed if the small intestine could be saturated for 4.5h. In one version the derivation uses an absorption rate constant (KA) (Equation 4) [79] and another uses an estimate of the effective human jejunal permeability (P_{eff}) (Equation 5)[80]:

$MAD = S \times KA \times V \times T$ (Equation 2)

$MAD = P_{eff\ human} \times S \times A \times T_{si}$ (Equation 3)

Where S is the solubility, V the intestinal fluid volume (250 mL), A is the absorption surface area (7.54×10^4 cm^2) and T_{si} the transit time (3.32 hours) for the absorption site [79, 80]. This model links solubility with dose, permeability, and physiological parameters of the GI tract to demonstrate that as the dose increases, the required solubility must also increase to achieve adequate drug exposure.

Despite these attempts to predict the impact of drug solubility on absorption, there are many exceptions that result from more complex absorption behaviours that might be particularly important for low solubility drugs. For example, some drugs classified as having low solubility manage to maintain supersaturation in the intestinal fluids meaning that they are still well absorbed [12]. Other behaviours such as improved solubility in specific physiological conditions like FaSSIF (Fasted State Simulated Intestinal Fluid) and FeSSIF (Fed State Simulated Intestinal Fluid) may also hamper solubility impact predictions.

1.3.4. Solubility in Different Solvents

Since aqueous solubility not always reflects the actual solubility of drugs, it is important to find other alternatives that could better represent the solubility of drugs in the GI tract. The best way of doing this is by replicating the composition of the GI media. A huge effort has been expended to replicate the in vivo environment during solubility assessment and the result of these efforts were four levels of simulation of luminal composition ranging from simple approaches to more complex [81]. The level of complexity necessary for solubility

assessment increases during drug development to ensure that the measurements are appropriate for the product's risk assessment [51].

Level 0 media are formulated to replicate GI pH consisting mainly of pH buffer systems [81].

Level 1 media are formulated to replicate GI pH and buffer capacity. The GI tract is buffered by bicarbonate within anaerobic conditions, which are extremely difficult to conduct at laboratory conditions [81]. Therefore, in order to mimic these conditions *in vitro*, phosphate buffers are typically used because they offer ease of use, stability, and reproducibility in routine laboratory measurements. There is evidence that bicarbonate buffers may better reflect in vivo conditions for certain drugs; however, due to the practical challenges in their routine laboratory use, they are not widely adopted [51].

Level 2 media are formulated to replicate pH, buffer capacity, osmolality and solubilisation capacity using bile salts, dietary lipids and digestion products and are thus more complex [81]. These media include bile salts which are natural surfactants and can improve the solubility of low solubility drugs [82]. These amphiphilic molecules can form colloidal structures when in aqueous environments called micelles, in which drugs can associate and improve their solubility. In biorelevant media the bile salts form colloidal structures with phospholipids that include micelles that can enhance drug solubility [51, 82, 83]. These micelles, however, are only formed above bile salts' critical micelle concentration which indicates that small changes in the media composition can have an impact on the solubilisation ability [83]. Simulated intestinal fluids (SIF) and simulated gastric fluids (SGF) were formulated for this level of testing and different recipes were proposed with different media compositions [84-86]. The most common and commercialised versions are FaSSIF, FeSSIF and fasted state simulated gastric fluids (FaSSGF). The composition of these media is presented at Table 1.

Level 3 media are formulated to build on level 2 media and incorporating other biorelevant ingredients such as proteins and enzymes [81].

Given the variability and complexity involved in replicating the GI environment, particularly in the fed state, relying solely on these solubility increases for drug development can be a risky strategy. Therefore, careful consideration and selection of the appropriate level of biorelevant media are crucial to accurately predict in vivo drug behaviour and ensure the success of the drug development process [51].

Table 1. **Media compositions of the most commonly used simulated intestinal fluids**

	FaSSIF	FeSSIF	FaSSGF
Bile salt (taurocholate) mM	3	15	0.08
Phospholipids mM	0.75	3.75	0.02
Sodium ions	148	319	34
Chloride ions	106	203	59
Phosphate ions	29	-	-
Acetic Acid	-	144	-
pH	6.5	5	1.6

Note: Fasted state simulated intestinal fluid (FaSSIF), fed state simulated intestinal fluid (FeSSIF) and fasted state simulated gastric fluid (FaSSGF). Adapted from [81].

1.3.5. Solubility in Gastric and Intestinal Fluids

Human gastric and intestinal fluids, particularly human intestinal fluid (HIF), are critical for accurately quantifying gastrointestinal solubility and understanding drug absorption in the body [87]. HIF is considered the most relevant fluid for these studies because it closely mimics the conditions drugs encounter in the human gastrointestinal (GI) tract [88]. However, the collection and use of HIF present significant challenges that limit its practicality in routine drug solubility studies [60, 88, 89].

The process of collecting human intestinal and gastric fluids involves several logistical challenges that make it difficult to use these fluids routinely in drug solubility studies [60]. Several methods have been developed to collect HIF and assess drug solubility within these fluids, providing insights into the impact on oral bioavailability [90-93]. However, differences in collection protocols and storage conditions can further alter the fluid's characteristics over time and contribute to even more variability [60]. The collection process requires human volunteers, often involving invasive procedures like intubation to obtain fluid samples from different sections of the gastrointestinal (GI) tract. This process is not only uncomfortable for the volunteers but also time-consuming and costly, requiring specialised medical equipment

and trained personnel. Furthermore, the composition of these fluids can vary widely based on the individual's diet, health status, and the specific segment of the GI tract sampled although this variability can be somewhat normalised or reduced in solubility studies [60]. Due to these factors, obtaining human intestinal and gastric fluids is a complex and expensive process, making it impractical for routine use in drug solubility studies. Consequently, researchers often rely on SIFs as a more practical and consistent alternative (See Section 1.3.4).

1.3.6. Measuring Solubility

Measuring solubility is a crucial aspect of drug development, and various methods are employed depending on the stage of development and the specific needs of the study.

The most traditional and widely accepted method is the shake-flask method [94]. This approach involves adding an excess amount of the drug to a solvent, creating a saturated solution that is then shaken or stirred at a controlled temperature (usually 37°C) until equilibrium is reached, which is indicated by no further changes in solubility [12, 51]. This method provides an accurate measurement of equilibrium solubility, but it can be time-consuming, especially for poorly soluble compounds.

Another method is the intrinsic dissolution rate (IDR), which measures the rate at which a drug dissolves from a constant surface area under specific conditions, including pH and ionic strength of the solvent [51]. Unlike the shake-flask method, which focuses on equilibrium solubility, the IDR provides insights into how quickly a drug can reach its saturated solubility [51]. It is crucial to make the distinction between IDR and dissolution testing, as the IDR relates to the drug substance itself rather than the formulated product.

Kinetic solubility is another method often used in the early stages of drug development, particularly in high-throughput screenings. This method involves adding a drug in DMSO solution to aqueous buffers to measure solubility before the drug reaches equilibrium[12]. Kinetic solubility can help identify poorly soluble compounds early in the development process, but it tends to overestimate solubility compared to equilibrium methods, especially for highly crystalline substances [95]. Non-crystalline forms in early phases can show solubility differences up to 100-fold compared to crystalline forms [96], and cosolvents like DMSO may inflate solubility estimates [97]. Variations in assay conditions further reduce comparability.

For poorly soluble compounds, achieving accurate solubility measurements can be challenging due to slow dissolution rates. Various strategies, such as increasing the surface area of the drug or using amorphous forms to induce temporary supersaturation, can help overcome these challenges [12, 51].

Each solubility measurement method has its strengths and limitations, and understanding these is vital for selecting the most appropriate method at different stages of drug development and accurately interpreting the results.

1.3.7. Precipitation Risk - Supersaturation

Solubility in a drug formulation is governed by a dynamic equilibrium between dissolution and precipitation processes. Equilibrium solubility is the maximum concentration of a solute that can dissolve in a solvent when the system has reached a stable balance [51]. However, in practical scenarios, especially when solubility-enhancing excipients or gastrointestinal fluids are used, there can be a temporary increase in solubility beyond this equilibrium level, creating a supersaturated solution [51, 98, 99]. This state is inherently unstable and carries a significant risk of precipitation, where the dissolved drug can revert to its solid form [98, 99].

In the GI tract, where drugs are absorbed, the risk of precipitation depends on the balance between the rate of drug absorption and the rate at which it might precipitate out of solution[100]. If a drug is absorbed quickly, the risk of precipitation decreases because the dissolved drug is removed from the GI fluids before it can precipitate [51]. However, if the drug remains in a supersaturated state for too long, the probability of precipitation increases.

Ideally, the highest solubility should coincide with the drug's presence in the small intestine, the primary site of absorption. In cases where solubility is slow to reach equilibrium it is critical that this peak occurs in the small intestine [51]. For drugs with slower solubilisation rates, adding solubility-enhancing excipients could speed up dissolution and help achieve higher concentrations in the GI fluids therefore improving absorption [101]. However, using supersaturated formulations is risky because controlling the rate of precipitation is challenging, and the precipitated form might be a different polymorph from the administered drug, complicating the solubility and dissolution profile [51, 100].

Supersaturation will be further discussed in Chapter 8.

1.4. Dissolution

Dissolution is a kinetic process that measures the rate at which a solute dissolves in a solvent. It differs from solubility (Section 1.3), which refers to the maximum amount of solute that can dissolve in a solvent at equilibrium [102, 103].

Dissolution typically follows first-order kinetics, where the rate of solubilisation starts rapidly due to a high concentration gradient between the solute and solvent. This rate decreases as the solvent becomes saturated, eventually plateauing at equilibrium solubility [102]. In biopharmaceutical contexts, complete dissolution may occur before reaching saturation, especially when the concentration of solute is far below the solvent's saturation point. The relationship between dissolution rate and saturation was establish by Noyes-Whitney [104]:

$$\frac{dC}{dt} = \frac{DA(C_s - C)}{h} \quad \text{(Equation 4)}$$

where $\frac{dC}{dt}$ is the rate of dissolution, D is the diffusion coefficient of the solute, A is the surface area of the solute, Cs is the saturation solubility, C is the concentration of the solute in the solution, and h is the thickness of the diffusion layer [104].

The Noyes-Whitney equation serves as the basis for understanding how various factors, such as agitation, temperature, and formulation strategies, can impact drug dissolution and, ultimately, bioavailability [84, 104]. It highlights that increasing the surface area of the solute (e.g., by reducing particle size) or enhancing the concentration gradient (e.g., by removing dissolved drug through absorption) can significantly increase the dissolution rate, which is crucial for drugs with low solubility [102].

Drug formulation also plays a crucial role in dissolution. Excipients, which are inactive ingredients in a drug formulation, can significantly influence the dissolution process (e.g., surfactants can enhance drug wettability, which aids in the dissolution of hydrophobic drugs). Many factors that influence drug solubility also significantly impact its dissolution [102, 103]. Both processes are affected by the drug's physicochemical properties, including particle size, crystalline or amorphous form, and pKa, as well as environmental factors of the GI tract like pH and the composition of gastrointestinal fluids. This relationship demonstrates how solubility and dissolution work together to shape a drug's bioavailability and therapeutic performance, emphasising their fundamental role in biopharmaceutical studies.

1.4.1. Dissolution and Drug Absorption

Dissolution plays a critical role in absorption, particularly when drugs are delivered in solid dosage forms like tablets or capsules [103]. When a drug product is taken orally, it first undergoes gastric emptying and intestinal transit, which delivers the drug to the site of absorption in the GI tract. At the absorption site, the dissolution step is essential because only dissolved drugs can permeate the intestinal membrane, enter the bloodstream, and ultimately reach systemic circulation [103].

If the dissolution of a drug is slow compared to other processes such as gastric emptying, intestinal transit, and membrane permeation, it becomes the rate-limiting step in drug absorption. This is particularly true for poorly soluble drugs, where the dissolution rate controls the overall rate and extent of drug absorption, directly impacting bioavailability. In these cases, even if a drug has high permeability and is metabolised efficiently, its therapeutic effectiveness may be compromised if it cannot dissolve adequately in the GI fluids [102].

The significance of dissolution in drug absorption was first highlighted by Nelson in 1957 [105], who established a link between the *in vitro* dissolution rate of theophylline salts and their blood levels when administered orally. This discovery underscored the importance of dissolution testing as a predictor of oral drug absorption and bioavailability and establishing dissolution as an essential test during drug development.

1.4.2. Dissolution Testing

As a key test in drug product development, dissolution analysis provides vital information about the rate and extent of drug release from a formulation. This test is particularly important for low solubility drugs, where dissolution can greatly influence pharmacokinetics, and for extended-release (ER) products, where the controlled release of the drug determines the available concentration for absorption [102, 103].

Through carefully designed dissolution tests, it is possible to optimise product performance, such as creating ER formulations that maintain a stable and prolonged plasma drug profile, reducing side effects, and improving patient adherence [102]. Moreover, dissolution testing serves as a critical tool for ensuring batch-to-batch consistency and assessing the impact of formulation composition and manufacturing processes [106].

Pharmacopeial dissolution testing is crucial for ensuring the quality, performance, and consistency of oral drug products. Regulatory guidelines have been established to standardise these tests, emphasising their importance for marketed drugs. For quality control, dissolution tests typically involve specified conditions, such as using a dissolution medium of 1000 mL of pH 6.8 phosphate buffer, and apparatus like the USP paddle or basket apparatus. Adherence to such protocols ensures that drug products meet regulatory expectations for consistent therapeutic performance, providing a benchmark for quality across production batches.

Although the ideal dissolution test balances clinical relevance and biorelevance without adding unnecessary complexity, multiple test designs are often employed during development to gain a deeper mechanistic understanding of the drug's release profile [102]. This topic will be further discussed in Chapter 8.

1.5. The Fasted and Fed States

The fasted state refers to the condition of the gastrointestinal tract following an overnight fast. During this state, the digestive system operates without the influence of recently ingested food or digestive products, making it a baseline for studying the body's natural gastrointestinal physiology. The fluid in the intestines during this time is considered representative of the body's normal state, free from the variables introduced by food intake.

The fed state occurs after the consumption of food and is characterised by significant changes in the composition and volume of gastrointestinal fluids. This state is marked by alterations in pH, surface tension, and osmolality, as well as variability in these factors depending on the type and content of the meal consumed. Factors such as the meal’s composition (whether high in fat, protein, or carbohydrates), its volume, caloric content, and even temperature can all influence drug absorption in this state, making it a more complex and variable environment compared to the fasted state [64].

Together, these physiological differences highlight the importance of considering fasted and fed conditions in drug development and biopharmaceutical evaluations. This topic will be further discussed in Chapter 7.

1.5.1. Food-Drug Interactions

Food-drug interactions significantly influence the safety and efficacy of oral medications. These interactions can be categorised as pharmacokinetic or pharmacodynamic depending on their type of influence.

Pharmacodynamic interactions occur when food components directly influence the drug's pharmacological effects. For example, caffeine can counteract the calming effects of beta-blockers due to its stimulant properties[107]. Another well known example of this type of food-drug interaction is the “cheese reaction” which is caused by the mediation between tyramine, a constituent of cheese or raw sausages, and inhibitors of the enzyme monoaminoxidase such as tranylcypromine [108]. Although these interactions can often be addressed through patient education or modifying the drug’s formulation, the diversity of dietary components and supplements presents challenges in predicting and avoiding such effects [109] [64].

Pharmacokinetic interactions influence the absorption, distribution, metabolism, or excretion of drugs. These interactions can be specific, such as the inhibition of enzyme CYP2D6 by certain spices, which slows the metabolism of antidepressants [110], or the inhibition of CYP3A4 metabolism by grapefruit juice which hampers the metabolism of drugs like cyclosporine and felodipine [111], or unspecific, involving generalised changes to gastrointestinal physiology. Unspecific effects include prolonged gastric emptying after fatty meals or increased bile salt concentrations that enhance the solubility of poorly water-soluble drugs. These physiological changes, induced by food intake, complicate drug bioavailability predictions, particularly for drugs with a narrow therapeutic index [64].

1.5.2. Food Induced Changes in GI Physiology

As mentioned in the previous section, the presence of food significantly alters the GI environment, affecting drug solubility, dissolution, and absorption mechanisms. In the fasted state, the concentrations of bile salts and lecithin, in GI fluids are relatively low, limiting the solubilising potential for lipophilic or poorly soluble drugs. In the fed state, these components

increase substantially, facilitating the formation of mixed micelles that improve drug solubility and bioavailability (See Section 1.3). Digestive enzymes are less active in the fasted state but are released in higher amounts during the fed state to aid in food digestion [64]. The pH of the GI tract also changes markedly after food intake. After eating, the buffering effect of food raises gastric pH to around 4–6. This pH elevation can alter drug solubility and dissolution behaviour, sometimes enhancing or reducing bioavailability depending on the drug's properties. Additionally, the pH in the small intestine may also rise slightly in the fed state, further influencing solubility dynamics [40].

Another significant change is observed in gastric emptying and intestinal transit times. In the fasted state, gastric motility is governed by the migrating motor complex, which moves undigested material through the GI tract in rhythmic phases. This results in relatively rapid gastric emptying and shorter intestinal transit, facilitating faster drug absorption but limiting the exposure of poorly soluble drugs to bile salts and digestive enzymes. After a meal, however, gastric emptying slows due to the physical presence of food, and intestinal transit time increases. These slower kinetics prolong drug residence time in the stomach and small intestine, enhancing opportunities for dissolution and absorption [40, 64].

The choice of drug formulation and administration is influenced by these physiological changes. Some formulations are designed for administration in the fasted state to avoid food interactions that could unpredictably alter absorption [112]. The fasted state conditions might also be ideal for immediate-release formulations since the rapid transit time and absorption will accelerate drug delivery. The fed state conditions and enhanced solubilisation may improve the solubility of poorly soluble drugs and thus their bioavailability [92]. Other examples such as extended-release formulations may be designed to exploit the slower transit times and more stable conditions in the fed state, resulting in more consistent drug release and absorption [85]. Understanding these differences is crucial for optimising drug delivery strategies and ensuring the desired therapeutic outcomes in varying physiological conditions.

1.5.3. Studying Food Effects

Regulatory authorities, such as the FDA [113] and EMA [114], mandate food effect studies to evaluate how the pharmacokinetic profile of a drug changes when taken with or

without food. These studies involve administering the drug to healthy volunteers in both fasted and fed states and analysing parameters like AUC, C_{max}, and T_{max} (See Section 1.1)[64]. These parameters provide insights into how food influences the drug's performance in the body.

The standardised protocols recommended by the FDA [113] and EMA [114] specify the use of a high-fat, high-calorie meal to ensure a comprehensive evaluation of food's impact. This "worst case scenario" meal includes foods such as buttered toast, fried eggs, bacon, hash-brown potatoes, and whole milk, collectively delivering 800–1000 kcal, with at least 500–600 kcal from fat. The drug is typically administered 30 minutes after the meal, accompanied by 240 mL of water. This timing reflects real-world scenarios where drugs are often taken shortly after eating [64].

To assess the food effect, the ratios of AUC and C_{max} between fed and fasted conditions are analysed within a 90% confidence interval. Acceptance ranges, usually set at 80–125%, determine whether the observed differences are clinically relevant [113]. These ranges may vary depending on the drug's therapeutic window, with broader or narrower ranges applied to accommodate safety considerations. Significant deviations from these predefined ranges indicate a notable food effect, categorised as positive (increased bioavailability) or negative (reduced bioavailability). These studies are essential not only for understanding the pharmacokinetics of the drug but also for determining practical usage recommendations. This topic will be further discussed in Chapter 7.

1.6. A new approach to simulated fluids

1.6.1. The “traditional” FaSSIF and FeSSIF media

As previously mentioned in Section 1.3.4, FaSSIF was developed to mimic the conditions of human intestinal fluids in the fasted state. The original version of FaSSIF [112], introduced in the late 1990s, was a significant advance in the field of biopharmaceutical research since it allowed for more accurate prediction of how drugs would behave in the human intestine compared to traditional buffer solutions. The first version of FaSSIF, was formulated to

replicate the fasted state conditions of the small intestine and its composition included a phosphate buffer to mimic the pH of the intestinal environment, typically around 6.5, and bile salts (sodium taurocholate) combined with lecithin to simulate the natural bile present in the intestine [112]. Recognising the need for further refinement, Galia *et al.*[112] and later on Jantratid et al [86], maintained the core components of the original FaSSIF but adjusted the concentrations to better match the physiological conditions observed in the fasted human intestine. Other versions were later introduced as part of ongoing efforts to enhance the biorelevance of simulated media (See Table 2).

The FeSSIF was developed to simulate the conditions of human intestinal fluid in the fed state. Similarly to FaSSIF, FeSSIF has undergone several iterations to improve its accuracy in mimicking the complex environment of the human intestine after a meal. The first version of FeSSIF (FeSSIF v1) was introduced alongside FaSSIF in the late 1990s as part of a broader effort to create biorelevant dissolution media [112]. The first version of FeSSIF was designed to reflect the higher bile salt concentration, increased lipid content, and altered pH observed in the intestine after food intake. The increased bile salt and lecithin concentrations were crucial for simulating the enhanced solubilisation capacity for lipophilic drugs in the fed state. Later versions, (See Table 3) also included sodium oleate to further enhance the medium's ability to dissolve lipophilic drugs, recognising that dietary fats contribute significantly to drug solubility in the fed state [86, 112].

While various SIF recipes have been developed to mimic gastrointestinal conditions, there is still debate over their accuracy. Drug solubility can vary widely based on the SIF composition and measurement methods used, making it challenging to determine the most accurate and representative media formulation. This variability complicates the selection of an optimal SIF for solubility assessments, highlighting the ongoing need for refinement to ensure that these media reliably simulate physiological conditions and yield predictive insights into drug behaviour in the gastrointestinal tract.

Table 2. **Composition of different fasted state simulated intestinal fluids found in the literature**

Component (mM)	Dressman *et al.*, 1998 (FaSSIF V1) [112]	Galia *et al.*, 1998 [112]	Pedersen *et al.*, 2000 [115]	Vertzoni *et al.*, 2004 [116]	Sunesen *et al.*, 2005 [117]		Jantratid *et al.*, 2008 (FaSSIF V2) [86]	Brinkmann-Trettenes and Bauer-Brandl, 2014 [118]
					Low	High		
Sodium TC	5	3		3	2.5	6.3	3	3
Lecithin	1.5	0.75	0.9	0.75	0.5	1.25	0.2	1.5
Sodium GG	-	-	3.7	-	-	-	-	-
NaH_2PO_4	29	28.6	50	28.66	29	29	-	32.9
Salt	220	103.3	150	106	-	-	68.62	105
NaOH	-	-	-	~13.8	-	-	34.8	98
Osmolarity (mOsmole)	280-310	270±10	-	270±10	-	-	180	-
Pancreatin (U/mL)	-	-	-	-	-	-	100	32
Tris/ Maleic Acid	-	-	-	-	-	-	19.12	-
pH	6.8	6.5	6.5	6.5	6.8	6.8	6.5	6.5

TC: Taurocholate; GC: Glycocholate

Table 3. **Composition of different fed state simulated intestinal fluids found in the literature**

Component (mM)	Dressman *et al.*, 1998 [112]	Galia *et al.*, 1998 [112]	Vertzoni *et al.*, 2004 [116]	Jantratid *et al.*, 2008 [86]	Kleberg *et al.*, 2010 [83]
Sodium TC	15	15	15	10	5-20
Lecithin	4	3.75	3.75	2	1.25-5
Buffer	Acetate	Acetate	Citrate	Maleate	Maleate
Salt (KCl)	0.19	0.20			
Sodium Oleate	-	-	-	0.8	0-45
Mono oleate	-	-	-	5	0-10
pH	5	5	5	5.8	6.5

TC: Taurocholate

1.6.2. Design of experiment studies

With many SIF recipes available all resulting in different solubility values and no agreement regarding which media better predicts in vivo solubility, it became essential to study the impact of individual intestinal components on drug solubility. A new approach to study the intestinal media components and how they contributed for drug solubility either individually or in combination with other media factors was proposed in 2015. This new approach consisted of a Design of Experiment (DoE) which is a statistical technique that helps to identify relationships between factors and responses. The primary goal of a DoE is to investigate the effects of multiple factors simultaneously on an outcome of interest, enabling researchers to identify and understand the complex interactions between these factors [119]. In a DoE, the factors represent the variables or parameters that are under investigation, and these are organised into different levels or settings. These levels can be adjusted systematically throughout the experiment, allowing for a comprehensive exploration of how changes in each factor influence the outcome [120].

The first set of DoE experiments created by the Strathclyde group focused on studying the impact of media composition variation on the solubility of fasted [121] and fed state [122].

- **Fasted DoE (Khadra *et al.*, 2015)**

The fasted DoE [121] used a quarter fraction factorial design with two extreme levels (low and high - Table 4) for seven components of typical SIFs: bile salts (sodium taurocholate), lecithin, sodium phosphate, sodium chloride, pH, pancreatin, and sodium oleate. The concentrations of components at both levels were based on literature values (See Table 2).

The experimental design involved 32 different media compositions, each measured in duplicate along with a centre point measured in duplicate. Statistical software (Minitab® 16.0) was used to analyse the main significant effects and 2-way interactions between components. The results showed that solubility range for all drugs were consistent with previous literature values from FaHIF or FaSSIF systems. Notably,

the solubility of some drugs, like zafirlukast and fenofibrate, displayed significant variability, which indicated sensitivity to different components, whereas drugs like griseofulvin and tadalafil had a condensed solubility range.

For the acidic drugs, five out of the seven media components tested significantly affected the acidic drugs solubility the exceptions being pancreatin and salt components. The study found that pH was the media factor with the most substantial impact on the solubility of acidic drugs, with a tenfold greater effect compared to other factors. Fatty acids, bile salts, and buffer components also significantly affected solubility which was consistent with earlier findings for drugs like indomethacin.

For basic and neutral drugs, six out of seven components significantly affected the basic and neutral drugs solubility, exception was for pancreatin only. Fatty acids and pH were the most significant factors, followed by bile salts and phospholipid with buffer and salt having lesser impact.

Additionally, the study identified 54 possible 2-way interactions between components, of which about one-third were statistically significant. Acidic drugs exhibited significant interactions primarily between pH and fatty acids or bile salts, and pH and buffer. Basic drugs had more significant interactions compared to acidic drugs, notably between pH and fatty acids, as well as pH and salt, and bile salts with fatty acids and buffer. Neutral drugs showed complex interactions involving pH with fatty acids, bile salts and salt, and bile salts with phospholipid and buffer.

Overall, the findings underscored the importance of considering multiple factors and their interactions when evaluating drug solubility in the fasted state, highlighting the complexity of replicating in vivo conditions in experimental settings.

Table 4. **Fasted DoE media compositions [121]**

	Lower value (mM)	Centre point (mM)	Upper value (mM)
Bile salt (taurocholate) mM	1.5	3.7	5.9
Phospholipids mM	0.2	0.6	1
Buffer	15	30	45

Salt	68	87	106
Enzyme (U/ml)	270	465	660
Fatty acid	0.5	5.25	10
pH	5	6	7

Bile salt: Sodium TC, Phospholipid: Egg phosphatidylcholine, Buffer: NaH_2PO_4, Salt: NaCl, Enzyme (U/mL): Pancreatin, Fatty acid: Sodium oleate

- **Fed DoE (Zhou *et al.* (2017))**

In a continuation of the fasted DoE study by Khadra *et al.* (2015) [121], Zhou *et al.* (2017) [122] extended the methodology to the fed state to explore its applicability and identify differences in drug solubility under these conditions. The study incorporated the same seven components as in the fasted DoE—pH, bile salts, lecithin, fatty acids, buffer, salt, and pancreatin— at higher concentrations while also introducing monoglyceride. This system also applied two levels - lower level and upper level- (Table 5) based on literature data (See Table 3). The fed state DoE included thirteen poorly soluble drugs, comprising five acidic (ibuprofen, indomethacin, phenytoin, valsartan, and zafirlukast), four basic drugs (aprepitant, bromocriptine, carvedilol, and tadalafil), and four neutral drugs (felodipine, fenofibrate, itraconazole, and probucol).

To maintain the statistical power of the original fasted DoE while accommodating the new component, the study used a D-optimal design, which required 92 samples (44 conditions, each in duplicate, plus 4 repeating centre points). This design allowed for a higher resolution of main effects but reduced the resolution of 2-way interactions. The results were compared with existing solubility data from fed state media such as FeHIF and fed SIF, showing that the solubility ranges found in the fed DoE were highly variable, sometimes spanning three logs, which was greater than the variability observed in the fasted DoE. The solubility results were consistent with literature values.

In terms of effects, bile salts had the most significant impact on solubility for twelve drugs, followed by pH, fatty acids, and lecithin, which significantly affected ten drugs each. For acidic drugs, pH was the primary factor influencing solubility, though its effect was diminished compared to the fasted state, likely due to higher surfactant

concentrations in the fed media. The effect of pH on acidic drugs was consistent with previous findings in human intestinal fluids. For basic drugs, bile salts were the most significant factor, followed by fatty acids, pH, and lecithin. Neutral drugs showed a mix of significant effects, with fatty acids having the greatest influence, followed by bile salts, pH, and lecithin, while salt had minimal impact. The findings emphasised the complexity of drug solubility evaluation in the fed state, demonstrating that media factors and their interactions significantly influence solubility. The variability in solubility results underscores the challenges of accurately replicating in vivo conditions with fed state media and highlights the need for careful consideration of component concentrations and their effects in experimental design.

Table 5. **Fed DoE media compositions [122]**

	Lower value (mM)	Upper value (mM)
Bile salt (taurocholate) mM	3.6	24
Phospholipids mM	0.5	4.8
Buffer	28.6	58.09
Salt	125	203
Enzyme (U/ml)	100	150
Fatty acid	0.8	52
Monoglyceride	1	6.5
pH	1	6.5

Bile salt: Sodium TC, Phospholipid: Egg phosphatidylcholine, Buffer: Maleic Acid, Salt: NaCl, Enzyme (U/mL): Pancreatin, Fatty acid: Sodium oleate, Monoglyceride: GMO

These DoE studies managed to yield unprecedented results that served as a foundation for further investigations. However, the large number of experiments involved in these studies (68 fasted; 96 fed) posed challenges for practical application. To address this, a DoE that reduced the number of required experiments while also

combining both fasted and fed states was developed [123]. This approach used a factorial DoE, which successfully reduced the experimental workload but made it impossible to distinguish between the fasted and fed states. To resolve this issue, a dual-level DoE design was introduced [124]. This design built on the previous approach, successfully reducing the number of experiments while also allowing for the differentiation between fasted and fed environments, thereby overcoming the limitations of the earlier study. A more concise method was later developed to further minimise the number of experiments while still accommodating component variation within Simulated Intestinal Fluid (SIF)[125]. This approach proposed a reduced dual-level DoE for both fasted and fed environments, reducing the number of experiments to 10 for the fasted state and 9 for the fed state [125]. It was effective in determining equilibrium solubility for BCSII drugs with a minimal matrix of solubility determinations and also identified the media factors most significantly affecting solubility. However, this outcome is constrained by the statistical limitations inherent in the small number of experiments conducted within the DoE.

This set of DoE studies highlighted that media composition plays a huge role in solubility prediction thus interindividual solubility variability should be expected in vivo since HIF compositions vary. Solubility in vivo will therefore most likely be a range and not a single value. Although successful in analysing the impact of the different components of the intestinal media on solubility, these DoE approaches were based strictly on statistical analysis of literature values and thus the ranges of solubility found do not reflect real-world population variability.

1.6.3. Multidimensional Analysis of Human Intestinal Fluids

In order to further refine existing SIF approaches, a multidimensional analysis of human intestinal fluids was performed [126]. This approach aimed to analyse and characterise the composition of HIF in both fasted and fed states in order to illustrate how the structured nature of traditional DoE does not adequately cover the complex variable space of HIF compositions. The goal was to address the limitations of SIF

systems that often do not account for the variability found in actual HIF by providing a statistical basis for more relevant SIF recipes that could better predict drug solubility and absorption in vivo.

The first part of the study consisted in analysing a previously published dataset [89] containing 152 measurements in the fasted state and 172 in the fed state, focusing on five key variables: total bile salt, total phospholipid, total free fatty acid, cholesterol, and pH. The data was collected from 20 volunteers and demonstrated complex, non-normally distributed data sets. The samples of HIF were collected by nasogastric catheter. The fasted state was achieved after an overnight fast of more than 12 hours and administration of 250 mL of water before sampling. The fed state followed the fasted sampling procedures, and the volunteers ingested 400 mL of Ensure Plus (a nutritional drink) and 250 mL of water after 20 minutes.

The five variables (total bile salt, phospholipid, total free fatty acid, cholesterol, and pH) were visually plotted in two-dimensional figures, with bile salts as a constant x-axis (Figure 8). The results revealed a cloud of data with an ellipsoid distribution where the concentrations of amphiphilic variables (bile salt, phospholipid, free fatty acid, and cholesterol) were positively correlated, indicating interdependencies among these components.

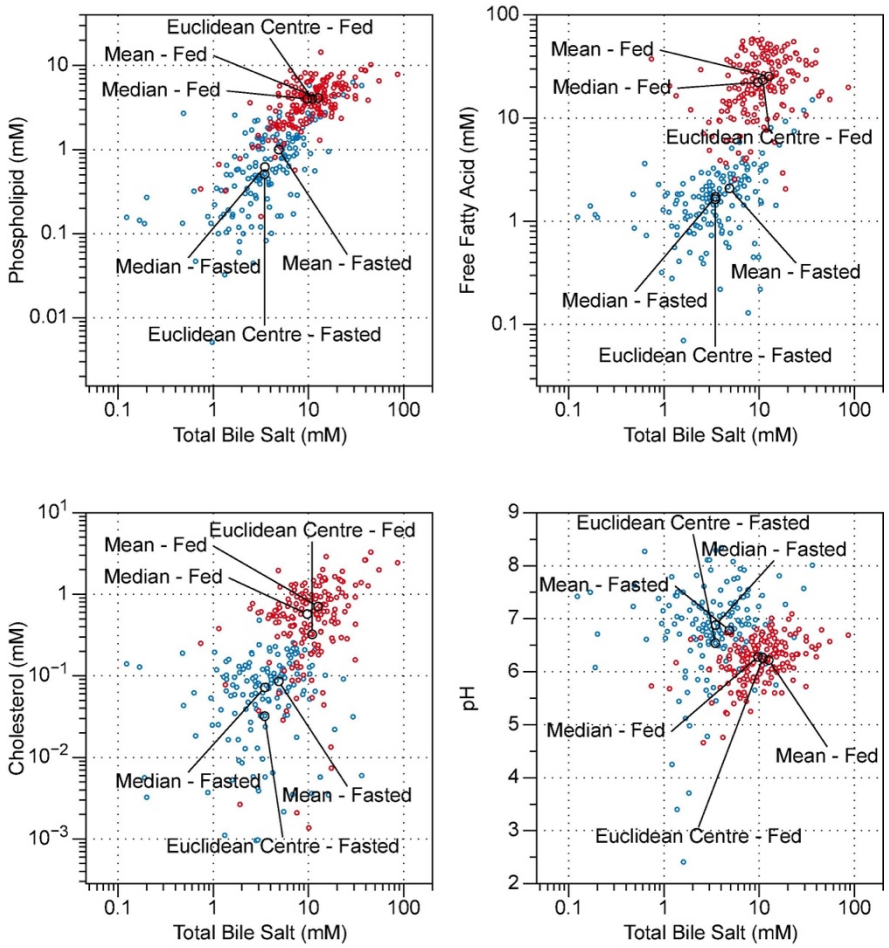


Figure 8. **Multidimensional Analysis data set: Fasted and fed matched data sets**

Note: red circles - fed state; blue circles - fasted state, statistical measures indicated by labels. Adapted from [126].

A Principal Component Analysis (PCA) helped in summarising the data from five variables into principal components that captured the largest variance in the dataset. The PCA also allowed to fit an ellipsoid to the multidimensional data cloud to visualise the composition space and identify boundaries enclosing the majority of the data. The ellipsoid allowed to determine statistical boundaries for SIF formulations that would more accurately reflect the characteristics of actual HIF.

Based on this analysis the study proposed eight biorelevant media compositions plus a centre point, that statistically captured over 95% of the compositional variability of HIF variation within the fasted and fed human intestinal fluid datasets. These compositions were selected to better reflect the range of conditions that might be encountered in vivo, thereby improving the relevance and accuracy of SIFs used in drug solubility and absorption studies.

By using these biorelevant media compositions, it is possible to mimic the actual conditions in the human intestine, leading to more predictive *in vitro* experiments that align more closely with in vivo outcomes.

Previous fasted state studies using these biorelevant media compositions demonstrated that fasted biorelevant media effectively replicates the fasted intestinal solubility envelope [127], providing solubility ranges with strong *in vitro*-in vivo correlations and more representative than previous SIF approaches [128]. They also identified solubility trends for different drug classes that could not be detected using single measurement approaches, emphasising the impact of pH and amphiphile media concentration on drug solubility [129]. Thus, the fasted multipoint approach was a successful attempt to improve biorelevance in solubility studies and showed such promising results that applying a similar methodology to the fed state could offer comparable advancements, paving the way for more robust models in fed state biopharmaceutical studies.

1.6.4. This Project

The hypothesis of this thesis is that enhancing the biorelevance of media used in biopharmaceutical tests, such as solubility and dissolution experiments, will improve the correlation with in vivo data. This, in turn, will lead to more accurate predictions of the factors influencing drug absorption, ultimately bridging the gap between *in vitro* testing and real-world outcomes.

1.6.4.1 Aims and objectives

The aim of this thesis is to evaluate the behaviour of a selected group of drugs (See Table 8) using fasted and fed state biorelevant media systems, derived from the multidimensional analysis of HIF [126]. Specifically, this research aims to: (1) apply fed state biorelevant media to measure solubility and compare the results with previously published data from other SIF and HIF approaches, (2) analyse and compare the drug behaviour in fed versus fasted conditions (3) perform additional biopharmaceutical tests including dissolution and supersaturation using both fed and fasted state systems.

This main aim is divided into six experimental chapters, organised as follows:

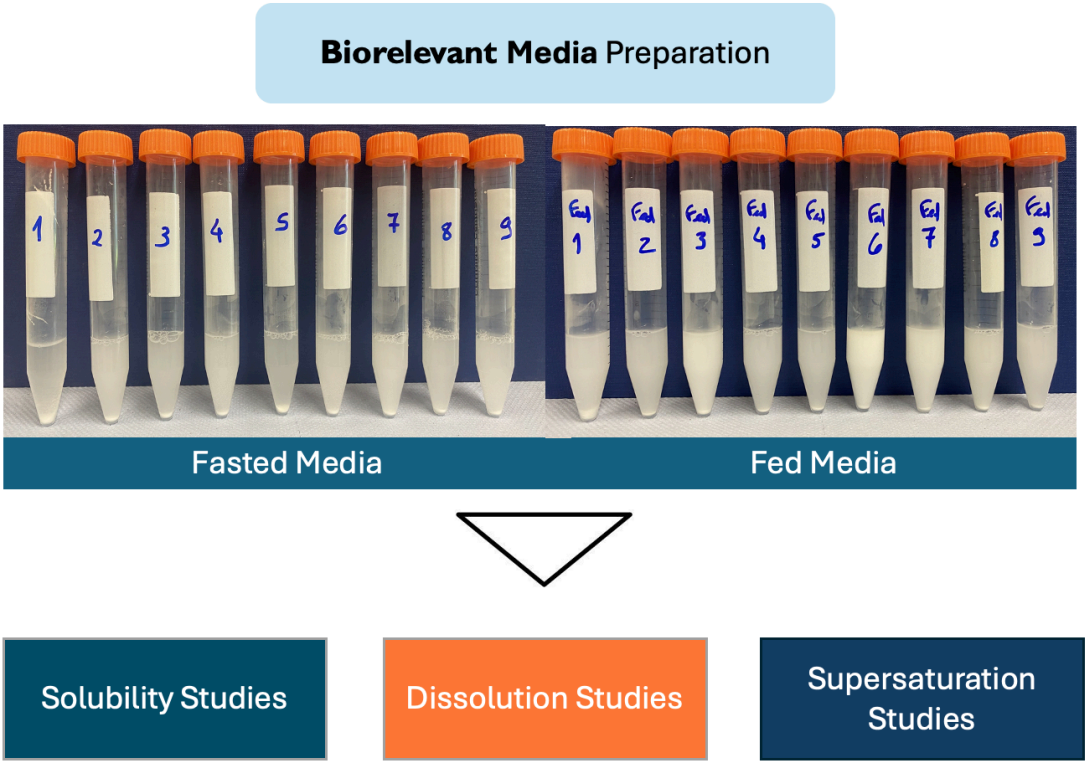
- **Chapter 3:** This chapter aims to compare two approaches (multidimensional analysis vs DoE [130]) for measuring drug solubility in simulated fed intestinal systems. The equilibrium solubility data was compared to the original fed DoE and to reduced experiment DoEs where appropriate data was available [123, 131, 132]. The objective was to determine the viability of using fed simulated media recipes derived using the multidimensional approach to measure drug solubility by comparing the data with the available literature. This chapter will provide a direct comparison to the approach applied to the fasted media systems [127]. Published in European Journal of Pharmaceutics and Biopharmaceutics, Vol. 177, 31.08.2022, p. 126-134.
- **Chapter 4:** This chapter aims to apply a fed state solubility range to the DCS grid and associated calculations, representing a novel approach in the literature. The solubility behaviour across the population was also assessed through a solubility frequency distribution. This chapter was published in European Journal of Pharmaceutics and Biopharmaceutics, Vol. 186, 31.05.2023, p. 74-84.
- **Chapter 5:** This chapter aims to analyse solubility behaviour to identify patterns that can define drug categories. Recognising such patterns could minimise the need for extensive simulated intestinal media measurements to establish a fed state solubility range. This chapter was published in European Journal of Pharmaceutics and Biopharmaceutics, Vol. 193, 31.12.2023, p. 58-73.
- **Chapter 6:** This chapter aims to establish an *in vitro*/in vivo intestinal solubility correlation by comparing Fa/Fe9SIF solubility data for various drugs to published Fa/FeHIF solubilities, aiming to evaluate the accuracy of the biorelevant system. This chapter was published in European Journal of Pharmaceutics and Biopharmaceutics, Vol. 199, 114302, 01.06.2024.
- **Chapter 7:** This chapter aims to develop a new tool for predicting food effects on drug absorption using simple solubility measurements and Solubility Limited Absorbable Dose (SLAD) calculations, and correlate findings with in vivo bioavailability and food effect predictions from literature.
- **Chapter 8:** This chapter aims to study the impact of media changes on the drug's IDR and correlate these findings with previous solubility data. Additionally, it

provides a comprehensive study of how biorelevant conditions influence drug supersaturation behaviour by using simulated intestinal media representing both fasted and fed states.

Chapter 2

Methodology

This section provides a detailed description of the preparation process for complex media representative of the fasted and fed states. It also outlines the methods used to investigate the solubility, dissolution, and supersaturation behaviour of a specific selection of drugs. The focus is on replicating physiologically relevant conditions to better understand the interplay between drug properties and gastrointestinal environments.



2.1. The Biorelevant Media

The fasted and fed media applied in this study resulted from the multidimensional analysis of HIF [126] described in the previous chapter (See Section 1.6.3). This approach captures the interindividual variability in media composition observed in real populations, improving SIF biorelevance and allowing for the investigation of media composition-dependent drug behaviours. Thus, instead of relying on a single, average composition, this study employs a series of nine media recipes for each state, including eight variations and a central point, representing a more comprehensive physiological range. To conduct the multidimensional analysis, the concentrations of measured components were combined and represented as a single variable. For instance, although six bile salt species were evaluated, their total concentration was consolidated into one value. In this study, the selected bile salt is sodium taurocholate, which comprises sodium, taurine, and cholic acid, with a total molecular weight of 537.7 g/mole. Since bile salt concentration is more influential on solubilisation than the specific species present [133], this simplification is also commonly applied to other SIF media. However, balancing these simplifications with the goal of accurately simulating native fluid remains a significant methodological challenge.

As in prior SIF media, lecithin (phosphatidylcholine from soybean) was selected to represent phospholipids, while sodium oleate was used for free fatty acids (FFAs). To achieve greater biorelevance, the media composition was refined to include cholesterol, a component absent in the previous DoE experiments but identified and quantified in the multidimensional analysis that originated the media recipes applied in this study. Incorporating cholesterol represents a purposeful effort to better replicate and understand physiological conditions, especially since it is absent in the composition of widely used FaSSIF and FeSSIF media.

Commercial FaSSIF and FeSSIF media purchased from Biorelevant.com Ltd was used for comparison with the media prepared.

Table 6 and Table 7 display the concentrations of media components of each media for the fasted (Table 6) and fed state (Table 7).

Table 6. **Fasted Media Compositions and FaSSIF**

Media	Bile Salts (mM)	Phospholipid (mM)	FFA (mM)	Cholesterol (mM)	pH	pH*TAC
1	1.060	0.160	1.040	0.010	6.640	15.07
2	11.45	2.480	2.880	0.380	7.120	122.4
3	3.560	1.180	1.040	0.060	5.720	33.40
4	3.400	0.330	2.880	0.090	8.040	53.87
5	3.350	0.310	0.870	0.170	6.620	31.11
6	3.620	1.250	3.430	0.030	7.140	59.48
7	2.270	0.960	1.010	0.080	7.340	35.01
8	5.330	0.400	2.960	0.070	6.420	56.24
9 (Centre)	3.460	0.520	1.640	0.032	6.540	36.96
FaSSIFv1	3.000	0.750	1.640	-	6.500	35.04

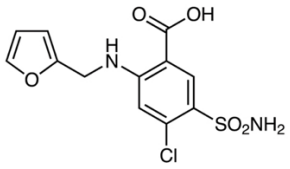
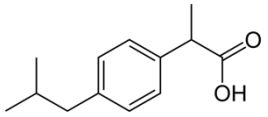
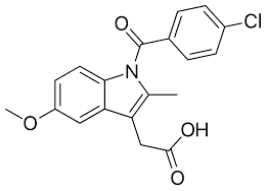
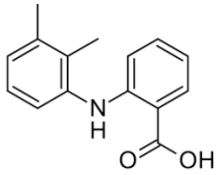
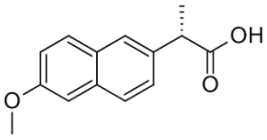
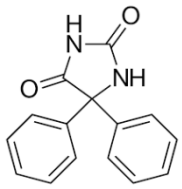
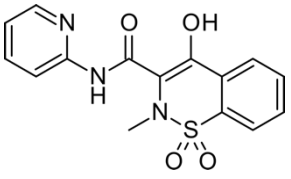
Table 7. **Fed Media Compositions and FeSSIF V2**

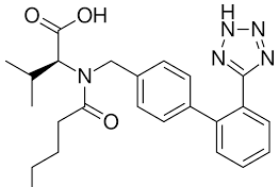
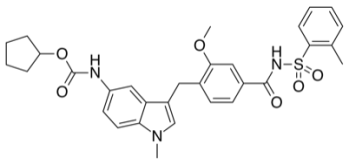
Media	Bile Salt (mM)	Phospholipid (mM)	FFA (mM)	Cholesterol (mM)	pH	pH*TAC
1	4.940	2.020	10.50	0.950	5.970	109.9
2	19.04	7.940	47.51	0.340	6.590	493.1
3	5.650	2.430	18.06	0.100	6.130	160.8
4	16.65	6.590	27.63	3.450	6.420	348.7
5	15.66	5.100	10.92	0.500	6.240	200.8
6	6.000	3.140	45.68	0.650	6.320	350.6
7	7.340	6.170	21.82	0.570	5.970	214.3
8	12.81	2.600	22.85	0.580	6.590	256.0
9 (Centre)	10.94	4.020	23.38	0.320	6.260	242.0
FeSSIFv2	10.00	2.000	0.800	-	5.800	74.24

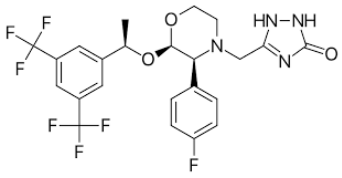
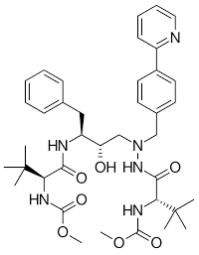
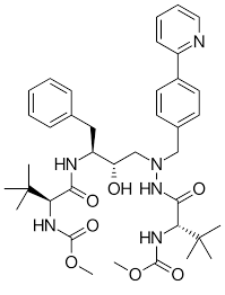
2.2. The Drugs

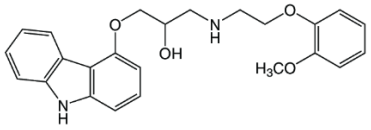
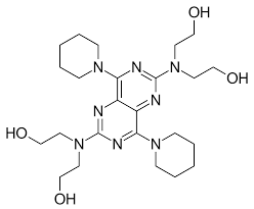
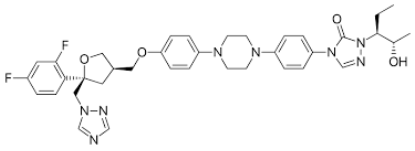
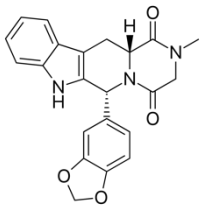
The drugs selected for this study were previously applied to DoE experiments [121-125] and in the DCS study [28]. In total 24 drugs were studied, although not all 24 drugs were used in the analysis of each chapter. The characteristics of these drugs are presented in Table 8.

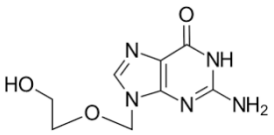
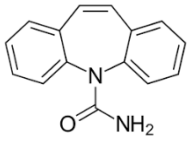
Table 8. **Physicochemical properties and molecular structures of drugs**

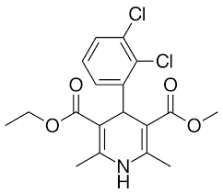
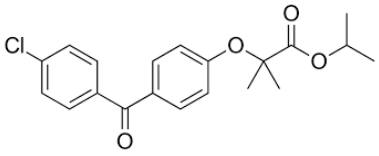
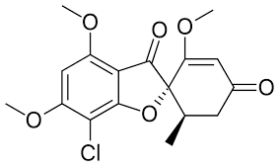
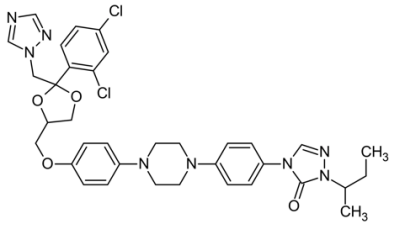
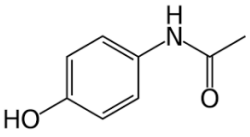
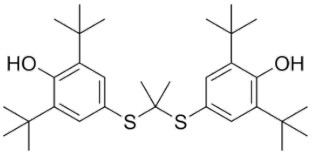
Acidic Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Furosemide	Diuretic	331	3.9	2.03	
Ibuprofen	Anti-inflammatory, analgesic	206	5.3	3.97	
Indomethacin	Anti-inflammatory	358	4.5	4.27	
Mefenamic Acid	Anti-inflammatory, antipyretic and analgesic	241	4.2	5.12	
Naproxen	Anti-inflammatory, analgesic	230	4.15	3.18	
Phenytoin	Antiepileptic, anticonvulsant	252	8.33	2.47	
Piroxicam	Anti-inflammatory, Antipyretic, analgesic	331	6.3	3.06	

Acidic Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Valsartan	Antihypertensive	436	3.9	1.5	
Zafirlukast	Anti-asthmatic	576	4.94	2.3	

Basic Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Aprepitant	Antiemetic	534	9.7	4.5	
Atazanavir	Antiretroviral	705	4.7	5.9	
Bromocriptine	Dopaminergic agent	655	6.68	3.2	

Basic Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Carvedilol	Antihypertensive	406	7.8	4.19	
Dipyridamole	Antiplatelet	505	6.2	3.77	
Posaconazole	Antifungal	701	3.6 & 4.6	4.6	
Tadalafil	Vasodilatory activity	389	3.5	1.7	

Neutral Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Acyclovir	Antiviral agent	225	2.52/ 9.35	−1.56	
Carbamazepine	Anticonvulsant, analgesic	236	–	2.45	

Neutral Drugs					
Compound	**Clinical Use**	**Molecular Weight (g/mole)**	**pKa**	**Log P**	**Structure**
Felodipine	Antihypertensive	384	–	3.86	
Fenofibrate	Antihyperlipidemic	361	–	5.2	
Griseofulvin	Fungistatic agent	353	–	2.18	
Itraconazole	Antifungal	706	-	5.66	
Paracetamol	Analgesic, antipyretic	151	-	0.46	
Probucol	Antilipidemic	517	–	11.3	

2.3. Media Preparation

2.3.1. Biorelevant 9 Media Recipes (Fasted and Fed)

Given the low concentrations of components like cholesterol and phospholipids (Table 6 and Table 7), particularly in the fasted state, precise measurement during media preparation posed challenging. To address this, a practical approach was adopted: stock solutions were prepared at concentrations 15 times higher than those listed in Table 6 for the fasted state and 2.5 times higher than those in Table 7 for the fed state. This method ensured accurate handling and incorporation of these components into the media, simplifying the preparation process while maintaining the required composition at final volumes.

Stock solutions of buffer (sodium phosphate monobasic monohydrate; 28.4 mM) and salt (sodium chloride; 105.9 mM) were prepared in water and used for both fasted and fed states.

- **Fasted stock solutions**

For the fasted state, nine stock solutions were prepared with 15 times the composition displayed in Table 6. Bile salts (sodium taurocholate), phospholipids (soybean lecithin) and fatty acid (sodium oleate) were combined in a flask and dissolved using chloroform, resulting in Solution A. Cholesterol was weighed in a separate flask and dissolved in chloroform to create Solution B. A 100 µl aliquot of Solution B was then transferred to Solution A, stirred, and the chloroform was evaporated using a stream of nitrogen gas until a dry film was formed. The lipid dry film was resuspended with water and transferred to a 5 ml volumetric flask, with the volume adjusted to completion using water.

- **Fed stock solutions**

For the fed state, nine stock solutions were prepared with the concentrations outlined in Table 7. Bile salts (sodium taurocholate) and phospholipids (soybean lecithin) were combined in a flask and dissolved with chloroform to create Solution A and cholesterol was weighed in a separate flask as Solution B. The same protocol as the fasted state was applied, and Solution A and B were combined, dried with nitrogen and resuspended as described above. Due to the higher concentrations of sodium oleate in the fed state media recipes, a separate stock solution of sodium oleate was prepared with water, sonication and elevated temperature. This solution was maintained at 50 ^{o}C to facilitate solubilisation.

- **Biorelevant 9 Media (Fasted and Fed)**

In order to match the desirable media concentrations in the fasted (Table 6) and fed state (Table 7) the final media was prepared by combining aliquots of the 15- and 2.5-times stock solutions of components with buffer and salt stocks at the desired final volume. To prepare 4 mL of fasted media, aliquots of 267 µL of media stock, buffer and salt stock were combined with 3.199mL of water. For the fed state (Table 9), a more complex preparation is necessary due to the separate FFA stock. For final volume, fed media stock should be combined with FFA, buffer and salt stocks and water to complete final volume. Because FFA has different concentrations in the 9 media, different aliquots are needed as shown in Table 9 for the preparation of 4mL of fed state media. The final volume can be adjusted according to the experimental needs however, it is important to maintain the same ratio of components when preparing different volumes in both states.

This media preparation protocol was followed and applied to all solubility chapters (Chapter 3, Chapter 4, Chapter 5, Chapter 6, and Chapter 7) using a final volume of 4mL. Volumes were adjusted for the preparation of fasted and fed media for dissolution and supersaturation experiments (Chapter 8), where bigger volumes were required.

Table 9. **Fed Media Preparation**

Media	Media Stock (mL)	FFA Stock (mL)	Buffer Stock (mL)	Salt Stock (mL)	Water (mL)
1	1.60	0.350	0.267	0.267	1.516
2	1.60	1.584	0.267	0.267	0.282
3	1.60	0.602	0.267	0.267	1.264
4	1.60	0.921	0.267	0.267	0.945
5	1.60	0.364	0.267	0.267	1.502
6	1.60	1.523	0.267	0.267	0.343
7	1.60	0.727	0.267	0.267	1.139
8	1.60	0.762	0.267	0.267	1.104
9 (Centre)	1.60	0.779	0.267	0.267	1.087

Note: Stock Media Volumes to prepare 4 mL of fed media

2.3.2. FaSSIF and FeSSIF Media

FaSSIF-v1 was selected for comparison with the biorelevant fasted media and FeSSIF-v2 for comparison with the biorelevant fed media. Both media were prepared according to the instructions provided by the manufacturer Biorelevant.com Ltd UK and using the manufacturer's fasted/fed buffer solution.

2.4. Solubility Experiments

Solubility experiments were performed for the selected group of drugs (Table 8) using the 9 fed state biorelevant media (Table 7). The results are displayed and discussed in Chapter 3, Chapter 4, Chapter 5, Chapter 6, and Chapter 7.

2.4.1. Measuring Equilibrium Solubility

Excess drug was weighed into nine Corning® centrifuge tubes and 4 mL of each of the 9 fed state biorelevant media were added. The pH of each tube was adjusted as

indicated in Table 7 (± 0.02 pH) using either KOH or HCl. The tubes were then shaken for one hour at room temperature, followed by a readjustment of the final pH as needed. All samples were placed in an orbital shaker (Labinco L28 Orbital Shaker) for 24 hours at 37 ^{o}C and 240 rpm. Following the incubation period, the tubes were inspected for the presence of solid drug and centrifuged at 10,000 rpm (Hettich Zentrifugen Mikro 20) for 15 minutes at room temperature. The supernatant was subjected to High Performance Liquid Chromatography (HPLC) analysis to determine the drug content. Three replicates were performed for each media point.

This method can be applied for both fasted and fed state media recipes.

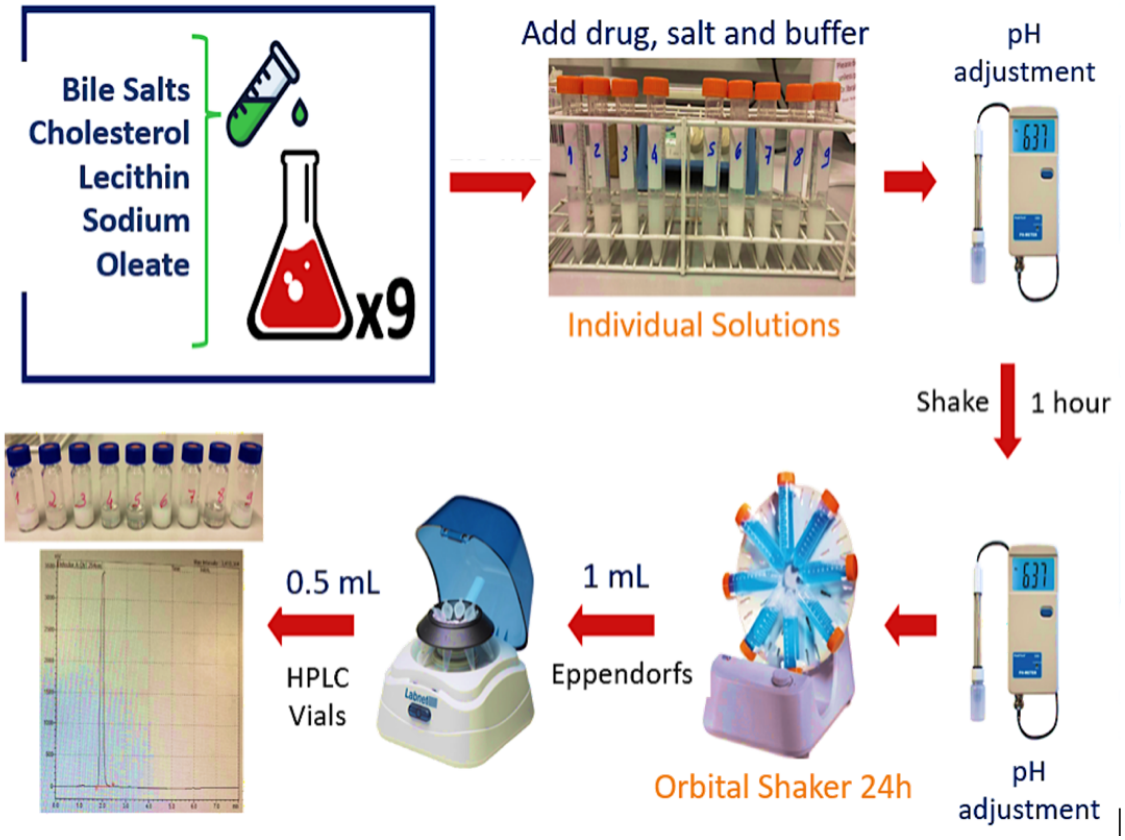


Figure 9. **Method to measure the equilibrium solubility of drugs using complex biorelevant media**

2.4.2. Analytical Techniques: High Performance Liquid Chromatography

HPLC is an analytical technique used to separate, identify, and quantify components within a mixture. HPLC is particularly well-suited for quantifying solubility in intestinal and SIFs due to its precision, sensitivity, and ability to handle complex mixtures. These fluids contain a variety of components, such as bile salts, phospholipids, enzymes, and digestion products, which HPLC can effectively separate from the drug of interest, allowing for accurate concentration measurement without interference.

This process involves:

1. Sample Injection: injection of a small volume of the liquid sample into the HPLC system. The sample contains the mixture of compounds that need to be separated and analysed.

2. Mobile Phase: The sample is carried through the system by a liquid solvent called the mobile phase. The mobile phase is pumped at high pressure through the system, which allows the sample to move through the column. The composition of the mobile phase can vary depending on the nature of the compounds being analysed, and it may consist of a single solvent or a mixture of solvents.

3. Column: The column is packed with a solid material called the stationary phase. The stationary phase is usually made of silica particles, but it can also be modified with different chemical groups to enhance the separation process.

4. Separation Process: As the sample travels through the column with the mobile phase, different components in the mixture interact differently with the stationary phase. Some components will interact more strongly with the stationary phase and move more slowly through the column, while others will interact less and move more quickly. This difference in interaction causes the components to separate as they pass through the column.

5. Detection: After separation, the components exit the column at different times and pass through a detector. The most common detector in HPLC is a UV-Vis detector, which measures the absorbance of the compounds as they pass through. The detector

generates a signal, typically shown as a peak on a chromatogram, which corresponds to the concentration of each compound.

6. Data Analysis: The detector's output is processed by a computer to generate a chromatogram, a graph that shows the detector signal (usually absorbance) versus time. Each peak on the chromatogram represents a different compound in the mixture. By comparing the retention time (the time it takes for a compound to travel through the column) and the area under the peak, it is possible to identify and quantify the components of the sample.

7. Quantification: The area under each peak is proportional to the concentration of the corresponding compound. By using calibration curves, which are created by running standards with known concentrations, the exact amount of each compound in the sample can be determined.

The technique's adaptability with different detectors (e.g., UV, MS, fluorescence) and column types enhances its ability to optimise separation and detection for a wide range of drugs. HPLC provides quantitative results, essential for solubility studies, where accurate drug concentration is crucial for assessing bioavailability. Its high sensitivity allows for the detection of drugs at low concentrations, often necessary in solubility studies. Furthermore, HPLC's reproducibility ensures consistent and reliable results across multiple experiments, making it a critical tool in drug development.

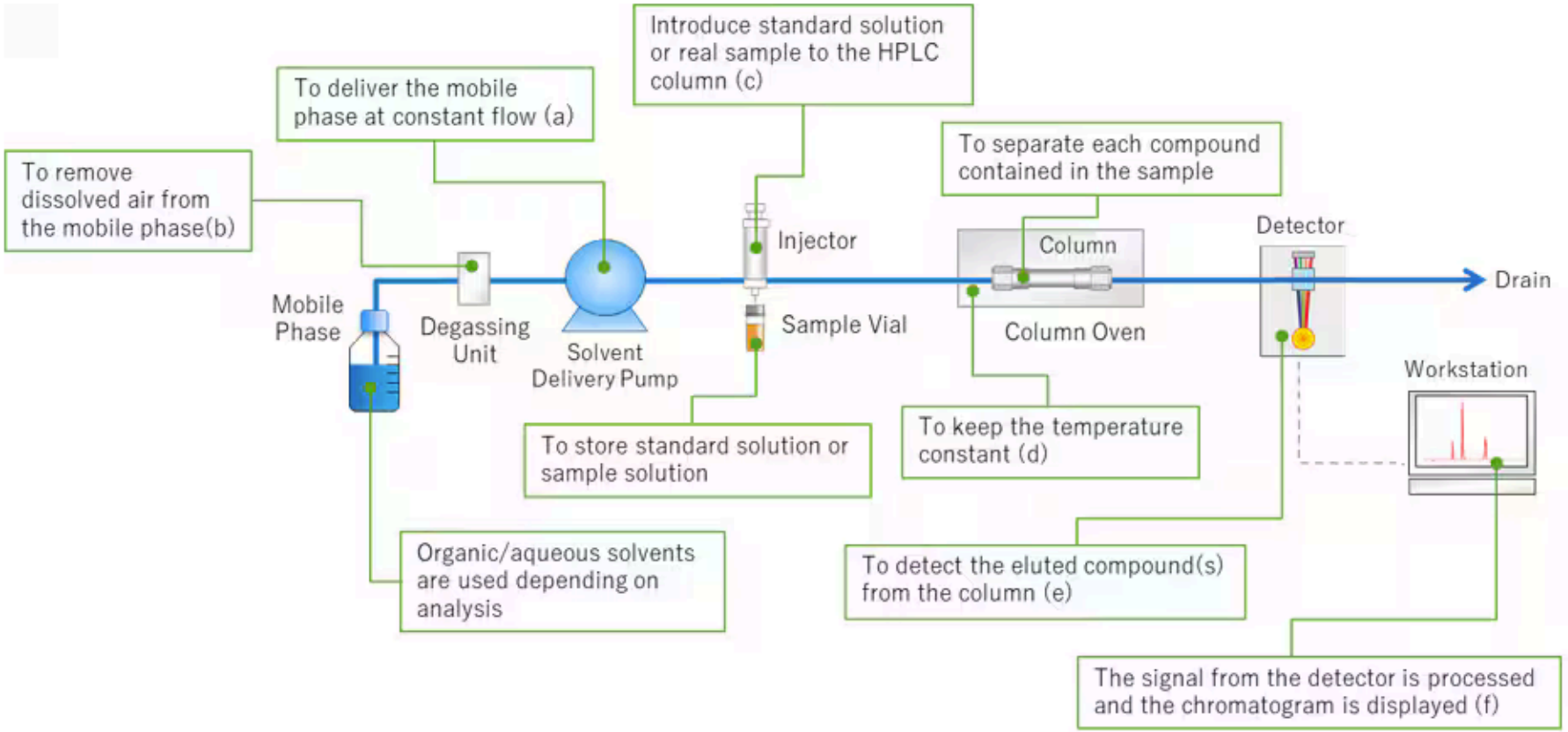


Figure 10. **HPLC flow diagram and component functions.**

Figure from [1]

2.4.2.1. HPLC analysis

HPLC analysis was performed using a Shimadzu High Performance Liquid Chromatography Prominence-I LC-2030C system using a gradient method for all the drugs (except bromocriptine) with the conditions specified in Table 9. This analytical method was previously applied to measure the concentration of a comparable group of drugs using SIF media in the fasted and fed state [121-125].

Calibration curves with a minimum of 6 points and 3 replicates, were constructed for each drug with correlation coefficients >0.99. The line´s equation was used to extrapolate the drug concentration.

Table 10. **HPLC Method Conditions**

Drug		Mobile Phase	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
Acyclovir	Fasted	**Mobile Phase A:** 10 mM Ammonium Formate pH 3 in H_20 **Mobile Phase B:** 10mM Ammonium Formate in ACN:H_20 (9:1 V/V)	0.5	10	254	1.52
	Fed			10	254	2.21
Griseofulvin	Fasted		1	10	291	1.5
	Fed			10	291	1.61
Paracetamol	Fasted		1	10	254	1.07
	Fed			10	254	1.08
Mefenamic Acid	Fasted		1	10	291	2.3
	Fed			10	291	1.71
Furosemide	Fasted		1	10	291	2.5
	Fed			10	254	1.07
Dipyridamole	Fasted		1	10	291	2.5
	Fed			10	291	1.60

Drug		Mobile Phase	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
Felodipine	Fasted		1	10	254	2.4
	Fed			10	254	2.60
Zafirlukast	Fasted		1	25	254	2.6
	Fed			10	254	2.53
Ibuprofen	Fasted		1	100	254	2.0
	Fed			10	254	2.06
Naproxen	Fasted		1	10	254	1.6
	Fed			10	254	1.5
Piroxicam	Fasted		1	20	254	1.07
	Fed			10	254	1.1
Phenytoin	Fasted		1	20	254	1.1
	Fed			10	254	1.0
Indomethacin	Fasted		1	10	254	2.1
	Fed			10	254	2.0
Carvedilol	Fasted		0.7	10	254	1.6
	Fed			10	254	1.6
Aprepitant	Fasted		1	50	254	2.27
	Fed			10	254	2.19
Tadalafil	Fasted		1	50	291	1.4
	Fed			10	291	1.49
Posaconazole	Fasted		1	10	254	1.9

Drug		Mobile Phase	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)
	Fed			10	254	2.1
Atazanavir	Fasted		1	10	254	1.7
	Fed			10	254	1.9
Carbamazepine	Fasted		0.7	10	291	1.9
	Fed			10	291	1.4
Fenofibrate	Fasted		1	10	291	3.0
	Fed			10	291	3.2
Probucol	Fasted		1	100	220	4.9
	Fed			10	254	4.4
Itraconazole	Fasted		1	10	254	2.4
	Fed			10	254	2.6
Valsartan	Fasted		1	10	254	1.18
	Fed			10	254	1.20
Bromocriptine	Fed	Isocratic method ACN and 0.1% w/v acetic acid (50:50 v/v)	1	10	291	0.58

Column: XBridge C18 5 µm 2.1x 50 mm, 30ºC

Gradient Start: 70:30 (A:B), 3 min 0:100, 4 min 0:100, 4.5 min 70:30

Total run time 8 min;

Drug	Mobile Phase	Flow rate (ml/min)	Injection Volume (µl)	Detection (nm)	Retention Time (min)

ACN- Acetonitrile

2.5. Dissolution Experiments

Dissolution experiments were conducted for griseofulvin, dipyridamole, and tadalafil (Table 8) using the fasted and fed biorelevant media outlined in Section 2.3. However, instead of using all nine media recipes, the dissolution experiments focused on media that represented the lowest and highest solubility for most drugs, capturing worst- and best-case scenarios while minimising experiments. For both fasted and fed states (Table 6 and Table 7), Media 1 had the lowest total amphiphile content (pH*TAC) and resulted in the lowest solubility values, while Media 2 had the highest pH*TAC and resulted in the highest solubility values (See Chapter 5). The results are displayed and discussed in Chapter 8.

2.5.1. Preparation of the Discs

Miniaturised discs of each of the three drugs chosen were prepared to undergo dissolution assays in the chosen fasted and fed media. The discs had approximately 5 mg of drug powder, 0.3 cm diameter and surface area of 0.071 cm^2. They were prepared using a Mini-IDR compression system (Heath Scientific, Milton Keynes, UK) for 2 min at 7 bars [134]. For each drug, three replicates were analysed with each disc prepared at similar conditions to ensure consistency.

2.5.2. Standard Curves

Before the dissolution study, standard curves for each drug were established using drug absorbance measured under the same conditions as in the drug study. The curves

were measured using the μDiss Profiler (Pion Inc., Billerica, MA) and the AuPro v.7.1 software. Stocks of drug were prepared with DMSO and aliquots with different volumes were added to 10 mL of biorelevant media. The volumes chosen were based on the concentration range that was intended for each drug and that it encompassed the drug's solubility value in each media. The dissolution media was maintained at 37 °C in the μDiss Profiler and stirred at 200 rpm during addition of the DMSO stock aliquots. Appropriate UV probe tips for the μDISS experiment were selected based on expected solubility and the strength of the chromophore of each compound; the probe tips (that determined absorbance path length) varied between 1mm, 2mm, 5mm and 10mm depending on the compound and the media used but kept the same for the different concentrations and calibration curve. For each drug, the standard curve was established based on six aliquots.

2.5.3. Dissolution Protocol

For the dissolution assays, the discs containing the drugs were inserted into rotating disc carriers and placed in the vials of the μDiss Profiler. To initiate the assay, 10 mL of biorelevant media was added to each vial and the stirring rate set to 100 rpm at 37 °C. The UV probes were set with the same conditions as the corresponding calibration curve including the same pathway size. The assay started at the exact moment that the media was first added to the vial. Data points were collected for 3 hours to represent intestinal transit time.

During this period, it is important to consider the potential for reaching the saturation solubility of the drug in the biorelevant media. If the solubility limit is reached, the media can become saturated, leading to precipitation of the drug and a plateau in the dissolution rate. Additionally, as the concentration of the drug in the solution approaches saturation, the system may move out of sink conditions, where the concentration gradient driving dissolution is no longer sufficient to maintain a high dissolution rate. Therefore, solubility was monitored and sink conditions maintained as much as possible during the assay including temperature control and constant stirring.

2.5.4. Calculation of Intrinsic Dissolution Rate

In order to compare the dissolution profiles of each drug in different media, Intrinsic Dissolution Rate (μg/cm^{2}min^{-1}) was calculated by the following equation:

$$IDR = \frac{dm}{dt}\frac{1}{A_{disc}} = Vk\frac{1}{A_{disc}}$$

where m is mass (μg), t is time (min), A_{disc} is the disc surface area (cm^2), V is the volume of the medium (mL), and k is the slope of the dissolution profile regression (μg/(min × mL)) obtained by plotting the dissolution profile using Prism 10 and fitting a nonlinear regression. The resulting slope was used to calculate the IDR for the compounds in each media.

2.6. Supersaturation and Precipitation Experiments

Precipitation experiments were conducted for felodipine, griseofulvin and tadalafil (Table 8) using the fasted and fed biorelevant media outlined in Section 2.3.

The media chosen for these assays corresponds to the lowest and highest solubility in both fasted and fed state (Media 1 and 2, Table 6 and Table 7) as stated above for dissolution (Section 2.5). The results are displayed and discussed in Chapter 8.

2.6.1. Initial Experiment

A stock solution of the API was prepared by dissolving each compound in dimethyl sulfoxide (DMSO) at a high concentration. The concentration of the stock solution depended on the compound's solubility in the media selected and was established as approximately 100 times the solubility in the media used. In μDiss vials, 10 mL of media and a crossbar magnetic stirrer were placed into the μDiss Profiler, with the temperature

set to 37°C and stirring speed maintained at 100 rpm. The black spectrum and 100% transmittance measurements were recorded.

To determine the highest concentration at which precipitation would occur, aliquots of the API stock solution (70 µL) were added to the media to increase the concentration of drug, and the absorbance was measured. The point at which the last addition of stock resulted in drug precipitation was noted as the supersaturation concentration (Cs,100%). Several repetitions were performed to ensure the reliability of the precipitation time, to ensure precipitation occurred within 10 minutes of the addition of Cs100%.

A standard curve was prepared by spiking aliquots of the stock solution into the medium, covering the equilibrium solubility and the determined Cs,100%. This standard curve was used to quantify the APIs concentration during the precipitation experiments.

2.6.2. Precipitation Experiment

After finding the Cs100% concentration for each drug, four different API stocks were prepared at concentrations of 100%, 87.5%, 75%, and 50% of the Cs100% [135].

At the beginning of each precipitation experiment, vials were filled with 10 mL of fresh media, the probes were calibrated, and the temperature was set at 37°C. The µDiss Profiler was set to record 3000 spectra with 5-second intervals. After initiating measurements, 200 µL of each API stock solution was added to respective vials, and the addition time was recorded. All experiments were continued for 60 minutes.

2.6.3. Data Analysis

The degree of supersaturation (DS) was calculated as the ratio of Cs to the equilibrium concentration (Ceq):

$$DS = \frac{Cs}{Ceq}$$

The equilibrium concentration was considered as the solubility of the drug in said media as previously measured in solubility studies and shown in Table 7.

The concentration-time curves were plotted using Prism 10 and a regression was fitted to each curve according to best fit. The induction time was considered as the time when the first drop of concentration is registered and therefore equivalent to X0. It was calculated using the plateau followed by one phase decay equation:

$$Y = IF(X < X0, Y0, Plateau + (Y0 - Plateau) * \exp(-K(X - X0)))$$

Where, X0 is the time at which the decay begins, Y0 is the average Y value up to time X0, plateau is the Y value at infinite times expressed in the same units as Y and K is the rate constant, expressed in reciprocal of the X-axis time units. If X is in minutes, then K is expressed in inverse minutes.

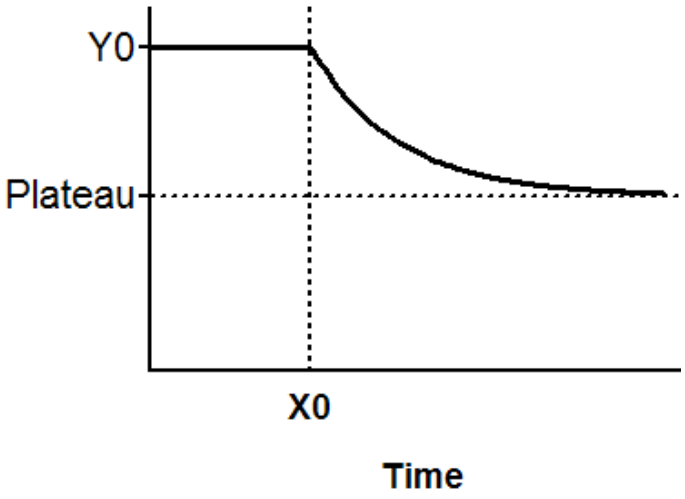
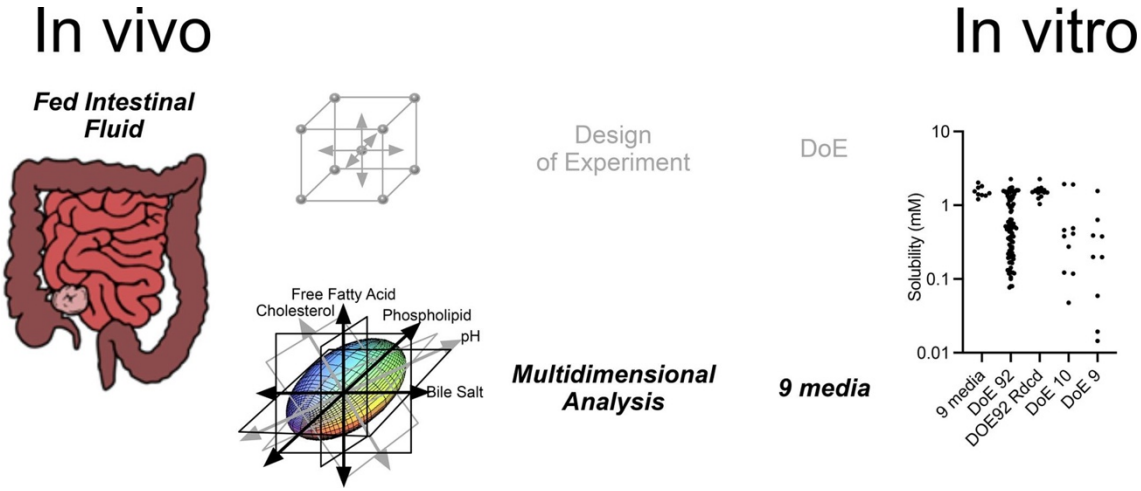


Figure 11. **Representation of the plateau followed by one phase decay equation**.

Note: Y0 corresponds to the drug concentration introduced in the system, X0 is the time when the first drop of concentration was registered indicating the start of precipitation and the Plateau corresponds to the lowest concentration registered after precipitation when the system is chasing equilibrium. Adapted from [136].

Chapter 3

Small Scale *in Vitro* Method to Determine a Potential Bioequivalent Equilibrium Solubility Range for Fed Human Intestinal Fluid



This chapter includes the full text of the published article in European Journal of Pharmaceutics and Biopharmaceutics. (Inês Silva, M., Khadra, I., Pyper, K., & Halbert, G. W. (2022). Small scale *in vitro* method to determine a potential bioequivalent equilibrium solubility range for fed human intestinal fluid. European Journal of Pharmaceutics and Biopharmaceutics, 177, 126-134. https://doi.org/https://doi.org/10.1016/j.ejpb.2022.06.005) including its introduction, methods, results, and conclusions.

For this work, I designed, analysed, and carried out all experiments and prepared the manuscript draft.

3.1. Introduction

The introduction of high-throughput screening systems allowed the development of thousands of new molecules through *in vitro* assays but resulted in an increase of compounds presenting low aqueous solubility [137, 138]. The pharmaceutical industry continues to prioritise oral drug administration because of its improved patient compliance and cost effectiveness [139]. However, a drug's solubility and therefore dissolution rate in gastrointestinal luminal fluids is a limiting step for this route, since a drug must be in solution before absorption [140, 141]. Poorly soluble drugs can lead to low oral bioavailability and compromise the drug´s therapeutic effect [142].

The BCS divides drugs into four classes by linking the drug's *in vitro* dose/solubility ratio with its in vivo bioavailability, highlighting that the rate and extent of drug absorption is controlled by the drug's solubility and gastrointestinal permeability [143]. Poorly soluble drugs (Class II and IV) are a challenge for the industry, requiring the development of new *in vitro* methods that allow gastrointestinal solubility assessment for these compounds.

When compared to simple aqueous solubility, gastrointestinal solubility is influenced by the presence of bile salts, phospholipids, food digestion products (e.g. monoglycerides and free fatty acids) and pH, all of which have the potential to enhance, depending upon physicochemical properties, drug solubility and dissolution [144]. Bile salts within the gastrointestinal tract form endogenous micelles, and digested fats present in food form mixed micelles (with phospholipids and bile salts), vesicles and colloids resulting in an increase in the solubilising capacity for poorly soluble drugs, especially after food intake [145-147]. Therefore, the higher concentrations of these intestinal components in the fed state when compared to the fasted state, can significantly impact the bioavailability of many compounds [148].

To measure gastrointestinal solubility, the most relevant fluid is HIF. However, the collection of HIF is a difficult and expensive process that requires human volunteers and varies depending on the protocols and storage conditions applied [140, 142, 149]. Multiple HIF studies from both fed and fasted states, highlight the problems with its collection, variability in different parts of the gastrointestinal tract and between individuals [150-152]. Thus, HIF is not a viable option for routine drug solubility studies.

The alternative to HIF is SIF that replicates *in vitro* the luminal gastrointestinal conditions for both fasted (FaSSIF) and fed states (FeSSIF) [140, 153, 154]. SIF utilises HIF components with known roles in drug solubility for example pH, bile salt, phospholipids and food digestion products such as free fatty acids and monoglycerides. FeSSIF media aims to mimic FeHIF and multiple recipes have been published [155] however, there is no consensus on the optimum recipe due to media solubility differences [130].

In this research group, a DoE study was conducted as part of the EU IMI Oral Biopharmaceutical Tools research programme [156], to statistically examine the influence of media components on equilibrium solubility in simulated fed media [130]. This DoE studied eight media components (bile salt, phospholipid, buffer, salt, pH, enzyme, fatty acid and monoglyceride) using a D-optimal design that required 92 experiments (see Table 11). The study identified the significant media components affecting solubility for the acidic (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast), basic (aprepitant, carvedilol, tadalafil, bromocriptine) and neutral (fenofibrate, felodipine, probucol, itraconazole) BCS II drugs investigated. Highlighting that for acidic compounds pH was the most significant media component, whilst for basic and neutral drugs the combination of pH and amphiphile (bile salt, phospholipid, free fatty acid and monoglyceride) concentration was significant. The study also identified various interactions between media components and unusual drug-specific solubility behaviour, emphasising that solubilisation in fed simulated media is a complex interplay of factors [157, 158]. However, this approach requires a large number of experiments and was not appropriate for routine application and early development studies. Therefore, reduced DoEs that combined both fasted and fed states in either 32 experiments [123] or a dual-level design with 20 experiments (10 experiments each in fasted and fed states) [131] were investigated (see Table 11). A further attempt to reduce the experimental load with 9 experiments for the fed state [132] was also studied. All the studies [123, 130-132] successfully quantified the drug's equilibrium solubility and were in general agreement with previous literature solubility values. However, the studies all utilised a DoE approach, which measures conditions that are statistically hypothesised to reflect the component variation within the experimental system or simulated fluid and not necessarily reflective of the natural composition. Thus, DoE approaches whilst capable of determining the impact of a media component on

drug solubility and its interactions with other components, they do not present a direct association to individual HIF sample compositions.

To circumvent the statistical construction of DoE systems a recent publication has studied HIF composition using data collected from fasted and fed HIF samples obtained from volunteers [159]. This study performed a multidimensional mathematical analysis of HIF composition that treated the fluid as a 5 dimensional system covering pH, bile salt, phospholipid, fatty acid and cholesterol concentrations [126]. These media components were based on the DoE results that indicated the importance of these components and their interactions for drug solubility. The 5 dimensional analysis identified 8 bioequivalent media compositions (see Table 7 and Table 11) that statistically characterised over 90% of the component variation within the HIF sample set in the fed state and calculated a centre point through a Euclidean approach in 5-dimensional space.

In this study we have applied the calculated fed state compositions from the multidimensional analysis to measure the equilibrium solubility of thirteen drugs (carvedilol, tadalafil, valsartan, fenofibrate, bromocriptine, phenytoin, itraconazole, indomethacin, probucol, ibuprofen, aprepitant, zafirlukast and felodipine) originally studied in the first fed state DoE [130]. The aim of this study is to compare the two approaches (multidimensional analysis vs DoE) for measuring drug solubility in simulated fed intestinal systems. The equilibrium solubility data was compared to the original fed DoE [130] and to the reduced experiment DoEs [123, 131, 132] where appropriate data was available. The aim is to determine similarities between the measured solubilities and the feasibility of utilising fed simulated media recipes derived using the multidimensional approach. This will provide a direct comparison to the approach applied to the fasted media systems [127].

Table 11. **Synopsis of Simulated Fed Media Conditions**

Study	pH	BS (mM)	PL (mM)	FFA (mM)	MG (mM)	Cholesterol (mM)	Buffer (mM)	Salt (mM)	Enzyme (IU/ml)	Number of Media	Statistical Design
Zhou 2017	5/6/7	3.6/13.8/24	0.5/2.65/4.8	0.8/26.4/52	1/3/6.5	ns	29/43/58	125/164/203	50/100/150	92[A]	D-optimal design
Ainousah 2017	5/-/7	3.6/9.3/15	0.5/2.1/3.8	0.8/13/25	1/5/9	ns	ns	ns	ns	10	1/16 Full Factorial Custom Design
McPherson 2020	5/-/7	3.6/15/24	0.5/2/4.8	6.6/20/33	1/5/6.5	ns	ns	ns	ns	9	Custom design
9 media This study	6/6.3/6.6	5/11/19	2/4/8	10/23/48	ns	0.1/0.3/3.4	ns	ns	ns	9	FeHIF 5D analysis

3.2. Materials and Methods

3.2.1. Materials

Sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl) and formic acid were purchased from Merck Chemicals Ltd. Lecithin S PC (phosphatidylcholine from soybean "98%") was purchased from Lipoid® Germany. Chloroform was obtained from Rathburn Chemical® and FeSSIF-v2 media from Biorelevant.com Ltd. Sodium phosphate monobasic monohydrate ($NaH_2PO^4 \cdot H_2O$) was from Fisher Scientific.

The active pharmaceutical ingredients carvedilol, tadalafil, valsartan, fenofibrate, bromocriptine, phenytoin, itraconazole, indomethacin, probucol and ibuprofen were purchased from Merck Chemicals Ltd. Aprepitant and felodipine were provided through OrBiTo by Dr R. Holm, Head of Preformulation, Lundbeck, Denmark and zafirlukast was purchased from Stratech Scientific Ltd.

The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

3.2.2. Methods

3.2.2.1. Stock media solutions for fed solubility experiments

As previously described in Section 2.3.

3.2.2.2. Equilibrium solubility measurement

As previously described in Section 2.4.

3.2.2.3. HPLC Analysis

As previously described in Section 2.4.2.1.

3.2.2.4. Data analysis

The data was compared using a non-parametric Kruskal-Wallis test with Dunn's multiple comparison correction using Prism 9 for MacOSX and only the comparisons represented in the figures were analysed. In order to calculate the significant factors influencing solubility, the media concentration values (Table 7) were used as input for a factorial custom DoE using Minitab®19.

3.3. Results and Discussion

3.3.1. Equilibrium Solubility Comparisons

The fed 9 media system equilibrium solubility results are in Figure 12, Figure 13, and Figure 14 for the acidic, basic and neutral drugs along with comparable data (where available) from previous fed DoE studies. A striking feature of all figures is that the initial 92 point DoE [130] has a greater solubility range than the other systems. Statistical comparison of the 9 media equilibrium solubility distribution with the 92 point DoE indicates that four out of thirteen drugs are statistically equivalent, which is in marked contrast to the fasted comparison [127], where ten drugs from twelve were equivalent to the fasted DoE study [160]. Comparison of the 9 media distributions with the 10 media DoE [131] or 9 media DoE [132] provides an improved correlation, with sixteen of twenty one comparisons statistically equivalent. This is similar, to the fasted where fifteen from eighteen were statistically equivalent. The 92 point fed DoE therefore produces a higher level of statistically different equilibrium solubility distributions when

compared to the 9 media system than either the 10 media or 9 media DoEs, which is different to the similar comparison of fasted DoEs [127].

The media component concentration values and statistical constructions analysed in these studies are not identical and a synopsis is presented in Table 11. The 92 point fed DoE examined eight components at three concentration values, and three (buffer, salt and enzyme) had no or negligible (when compared to the other components) impact on drug solubility [130]. These components were, therefore not examined in subsequent studies [131, 132]. The remaining components' (pH, bile salt, phospholipid, free fatty acid, and monoglyceride) concentration values in the smaller DoE studies [131, 132] is consistent between studies (with only minor differences), with the biggest difference the statistical design applied to determine the concentration values for each point. The 92 point DoE [130] utilised a D-optimal design, two concentration levels along with a centre point and required 44 media compositions dictated by the statistical design, measured in duplicate (centre point four replicates). To lessen the experimental load subsequent DoE studies [131, 132] reduced the number of media compositions and utilised either smaller fractional designs (10 media DoE) or custom media compositions based on literature data (9 media DoE). The difference between the DoE systems therefore, is not primarily controlled by media component concentration values but the number and variation of media compositions studied. The 92 point DoE with a larger number of statistically guided media compositions contains combinations of media components and concentrations that are not likely to be biorelevant (e.g. high bile salt concentration combined with low phospholipid and free fatty acid concentration) and or combinations that do not support or impair drug solubilisation [158]. The presence of these compositions is dictated by the statistical design and assists in calculating the impact of each component on drug solubility, but does not link directly to FeHIF composition.

The 9 media system compositions (Table 7 and Table 11) are based on a multidimensional analysis of FeHIF [126] not a DoE. The component concentrations between the 9 media and DoEs are different (Table 11 and Figure 15) especially the low DoE concentrations of bile salt, phospholipid, free fatty acid and pH. This arises from the limited media component concentration information available at the time of the 92 point DoE [149], resulting in component concentrations that are out with the 9 media

data cloud [126]. In the fasted media systems comparison these differences are not as pronounced (Figure 16) resulting in improved solubility determination equivalence between the DoE and multidimensional media [127]. The 92 point fed DoE therefore includes media component concentrations that are out with the FeHIF data cloud (Figure 15), and consequently due to the DoE design a wider variation in media component concentrations and combinations some of which are not likely to be biorelevant (see above), when compared to the 9 media system. This difference can explain the 92 point fed DoE's wider solubility range and lack of statistical equivalence to the 9 media system. Outlier compositions can be removed from the 92 point DoE (Figure 15) to form a reduced distribution consisting of fourteen solubility values, which based on Figure 15 are more likely to match the solubility behaviour of the 9 media measurements, see Figure 12, Figure 13, and Figure 14. A statistical comparison of the 9 media system with the reduced 92 point DoE improves the correlation with nine out of thirteen drug solubility distributions statistically equivalent. Highlighting that when the components concentrations lie within similar concentration ranges or limits the two systems are measuring the same solubility space.

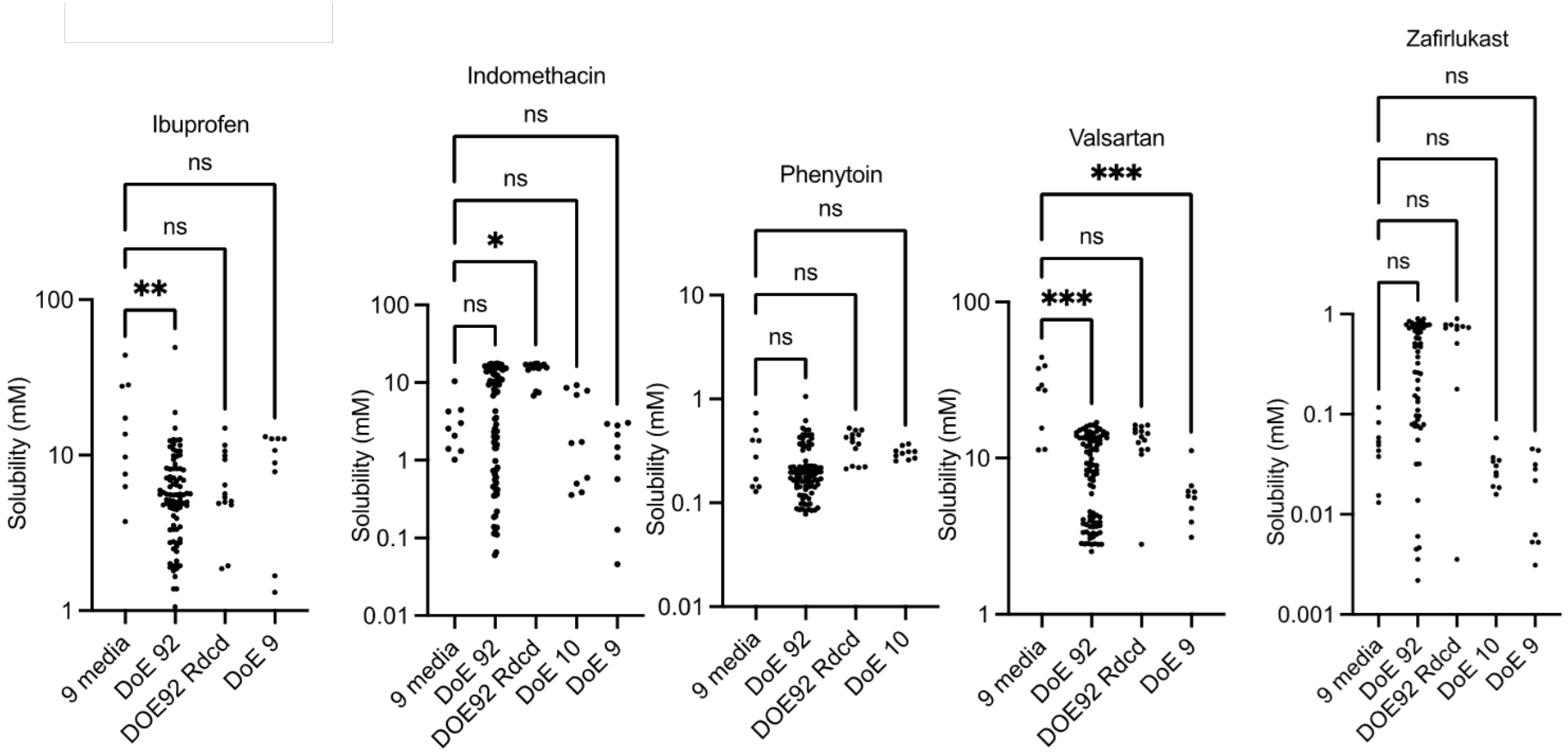


Figure 12. **Measured Equilibrium Solubility Distributions of Acidic Drugs**

Note: 9 media – this study; DoE 92 [130]; DoE92 Rdcd see text and Figure 15; DoE 10 [131]; DoE 9 [132]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0221; ** p = 0.0051; *** p = 0.0002.

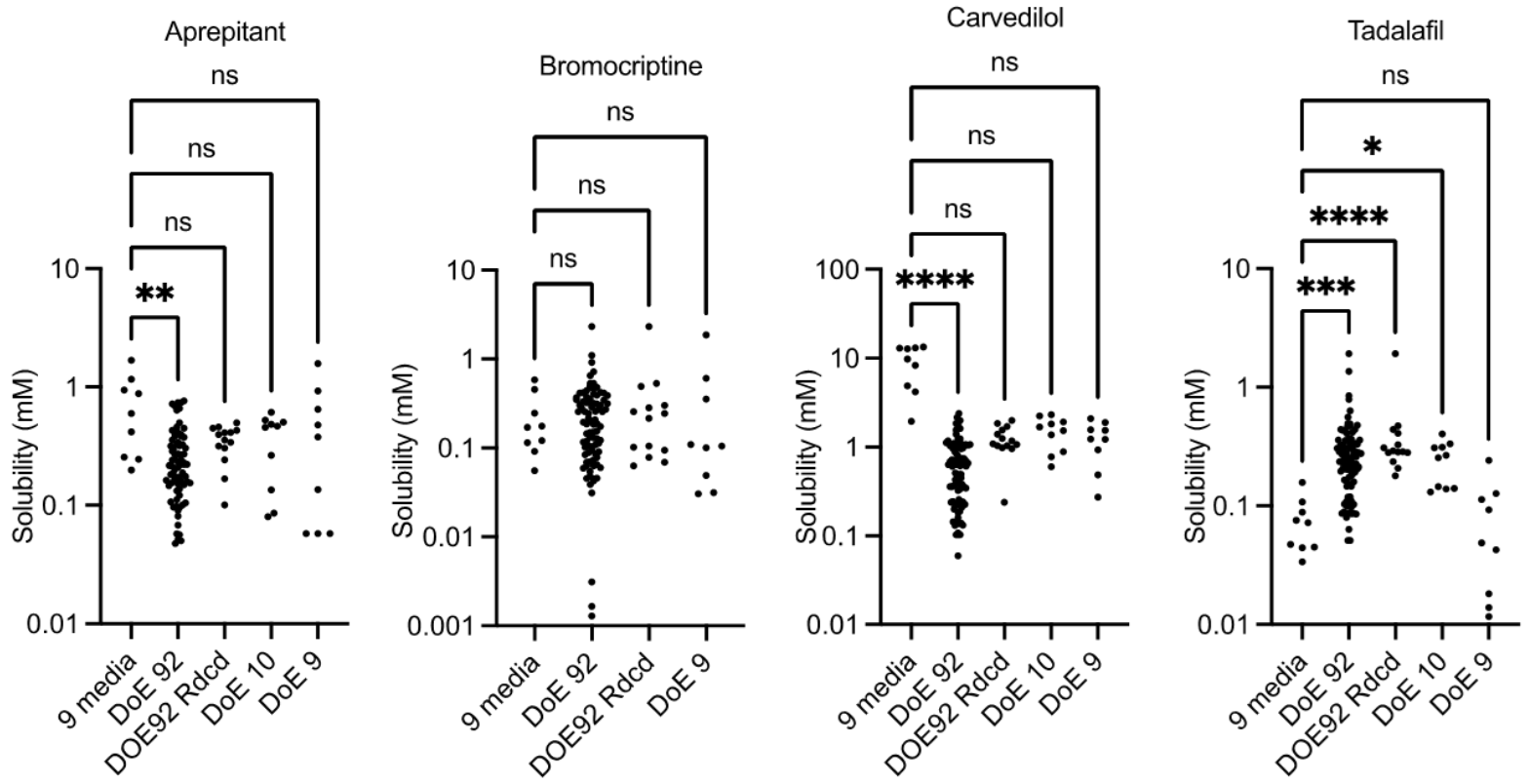


Figure 13. **Measured Equilibrium Solubility Distributions of Basic Drugs**

Note: 9 media – this study; DoE 92 [130]; DoE92 Rdcd see text and Figure 15; DoE 10 [131]; DoE 9 [132]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0191; ** p = 0.0029; *** p = 0.0003; **** p < 0.0001.

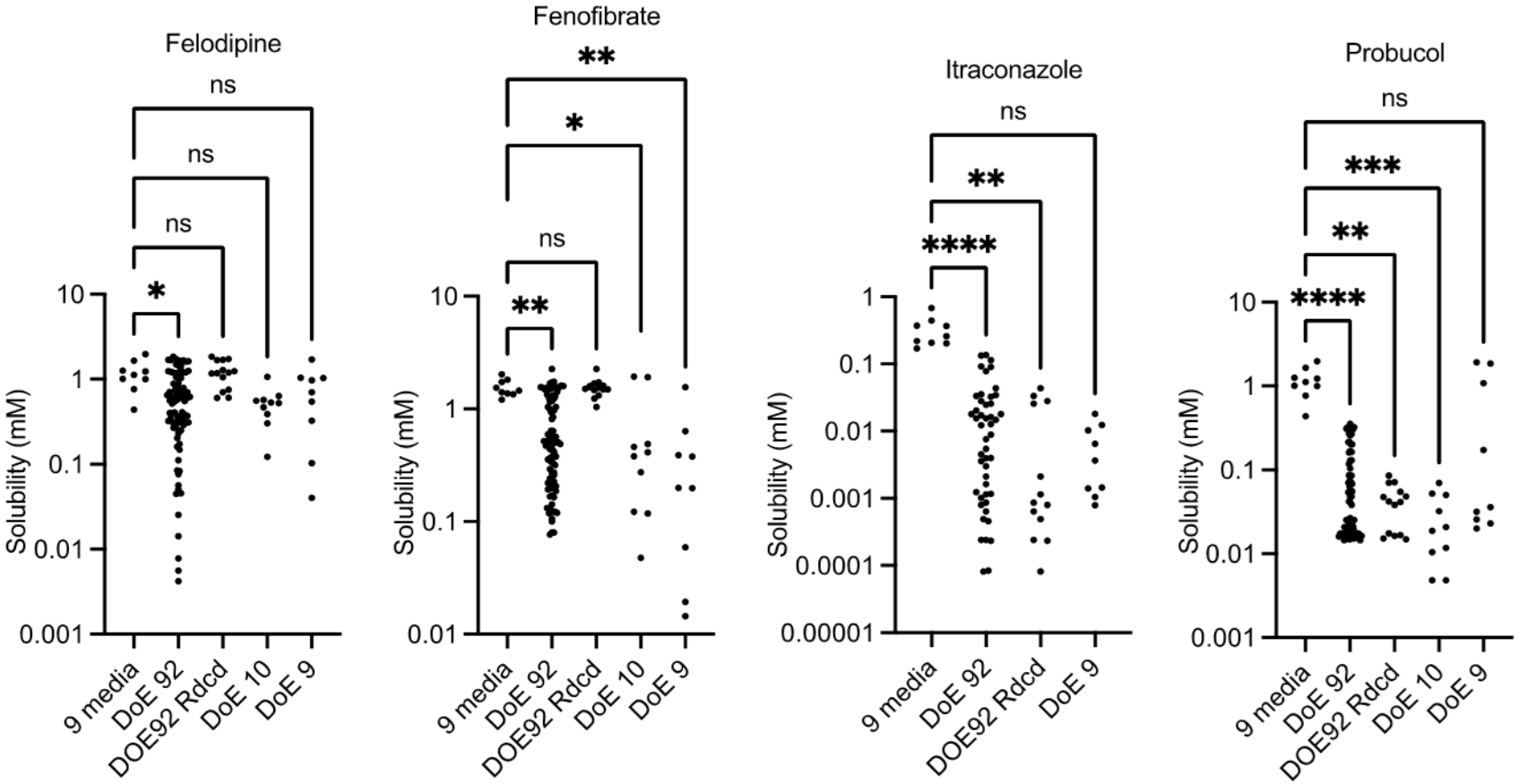


Figure 14. **Measured Equilibrium Solubility Distributions of Neutral Drugs**

Note: 9 media – this study; DoE 92 [130]; DoE92 Rdcd see text and Figure 15; DoE 10 [131]; DoE 9 [132]. Statistical comparison of 9 media against other systems, ns = no significant difference; * p = 0.0382; ** p = 0.0012; *** p = 0.0001; **** p < 0.0001.

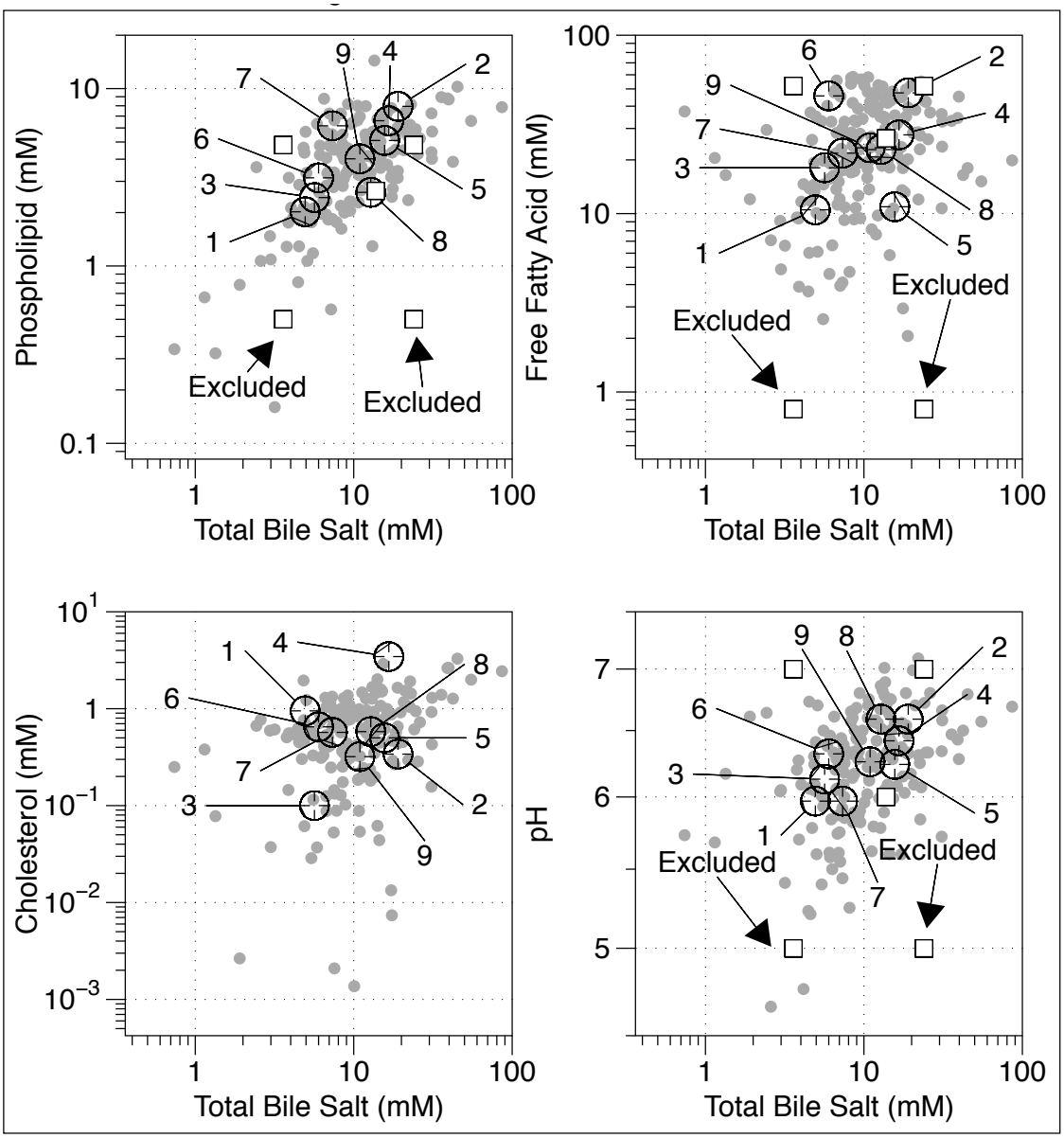


Figure 15. **Comparison Fed 9 Media Data Points, DoE 92 and Fed Data Cloud**

Note: ● Measured FeHIF data points taken from [126, 159]; ⊗ 9 media, this study points numbered as Table 7 ; ☐ DoE 92 points taken from [130], points excluded for DoE 92 Rdcd as indicated, see text.

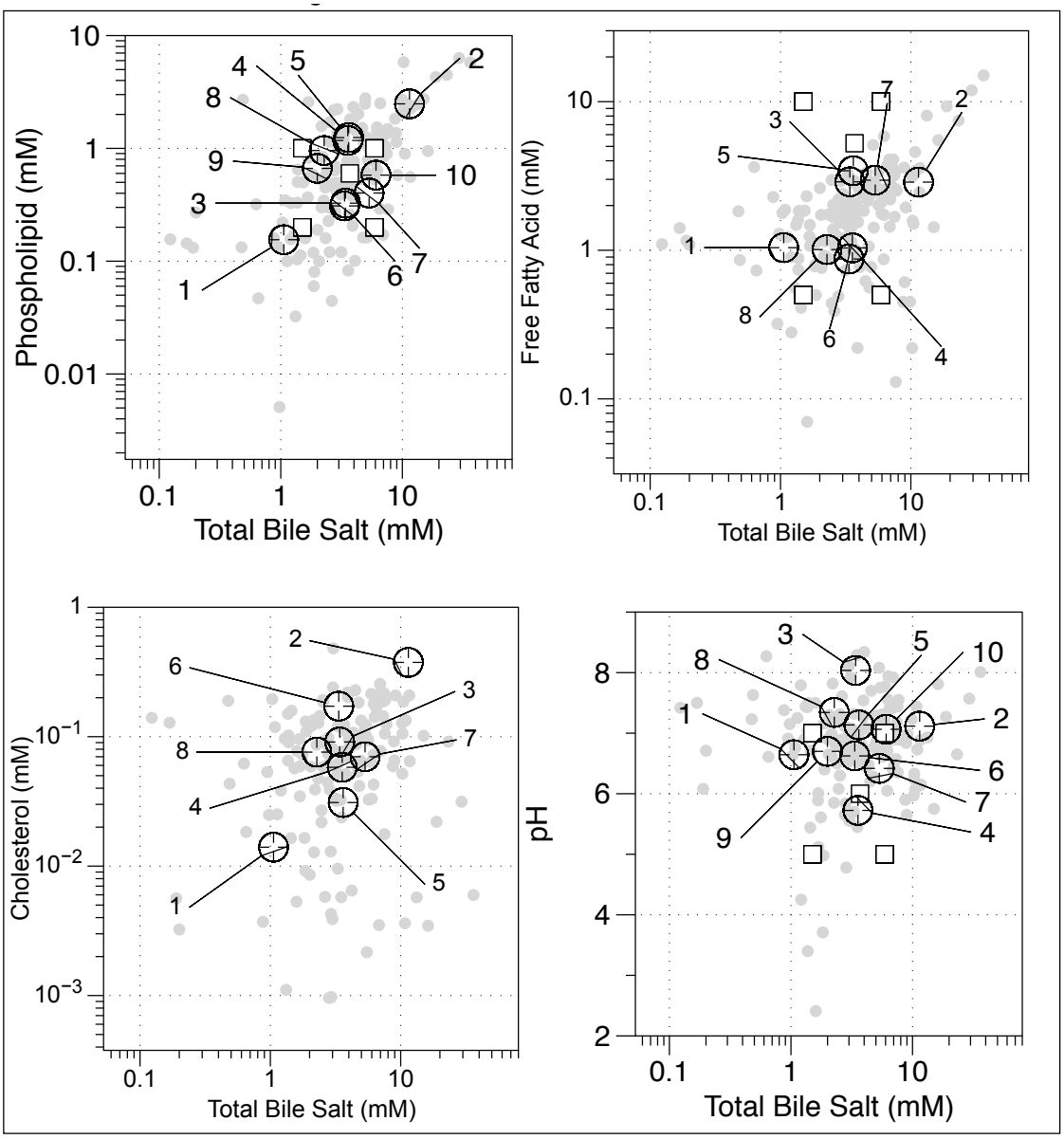


Figure 16. **Comparison Fasted 9 Media Data Points, DoE 66 and Fasted Data Cloud**
Note: ● Measured FaHIF data points taken from [126, 159]; ⊗ 9 media, taken from and numbered [127]; ☐ DoE 66 points taken from [160].

3.3.2. Solubility Multiple

The initial 92 point DoE highlighted that for some drugs solubility variability was up to three orders of magnitude [130]. For all drugs a solubility multiple was calculated by dividing the highest solubility with the lowest solubility measured in the system. A statistical comparison of the 9 media solubility multiple with the 92 point DoE (Figure 17) indicates that there is a significant reduction for all drugs studied (Figure 18), whilst a similar comparison with the reduced 92 DoE does not find a statistically significant difference. The results and discussion in the previous section in relation to component concentrations and compositions provides a rationale for this result. For the 9 media

system this indicates that the solubility distributions are lower and probably an improved estimate of FeHIF solubility than the 92 point DoE [130], which as discussed contains a large number of non-biorelevant systems.

There are some interesting variations within the solubility multiple values. It is noticeable for two drugs (zafirlukast and itraconazole) that the solubility multiple in the reduced 92 DoE is almost as large as the original indicating that these molecules are extremely sensitive to variations in media composition. Whilst for multiple other drugs (ibuprofen, indomethacin, phenytoin, aprepitant, and felodipine) the reduced 92 DoE has a lower multiple than the 9 media system. In these cases, the restricted media component concentration range (Figure 15) of the reduced 92 point DoE is likely to be responsible for this result (see next paragraph with respect to phenytoin), with the discussion in the previous section applicable.

The fasted 9 media system [127] revealed three drugs (phenytoin, tadalafil and griseofulvin (see also [157])) with small solubility multiples and a DCS study identified similar behaviour for acyclovir, paracetamol and carbamazepine [161] in the fasted state. A line drawn on Figure 18 (y = 5.71) at the solubility multiple for phenytoin in the 9 media system, indicates that tadalafil has a lower value along with valsartan and intriguingly all the neutral drugs (felodipine, fenofibrate, itraconazole and probucol). Valsartan was not studied in the fasted system and acyclovir, paracetamol and carbamazepine are not examined in this study. However, the low solubility multiple for tadalafil in combination with phenytoin indicates that this solubility behaviour for these drugs occurs in both fasted and fed states. This is worthy of further examination, as the bioavailability of drugs with this behaviour will not be influenced by intestinal fluid media composition. All the neutral drugs have smaller solubility multiples than phenytoin in the 9 media system, a result that is the reverse of the fasted state (itraconazole was not examined in the fasted study), where the solubility multiple value was larger. For fenofibrate for example the fasted 9 media solubility multiple is 7.65 [127] and in this fed study 1.67. For neutral drugs solubility is controlled by media pH and amphiphilic component concentrations [130] and this finding indicates that in the fed state with higher amphiphile concentrations there is a solubility variability smoothing effect. In a recent study [162] examining the bioavailability of fenofibrate in pigs after an FDA breakfast the AUC0-∞ standard deviation dropped from 24% of the mean value in the fasted state to 9% in the fed state. Multiple other factors for example metabolism or formulation could contribute to this difference, but the solubility finding reported in this study is worthy of investigation for drugs where a food effect is evident and *in vitro* models required [163]. The low

solubility multiple and possible solubility smoothing behaviour are interesting findings and drug dependent properties that are only revealed using a multiple media analysis [127, 161].

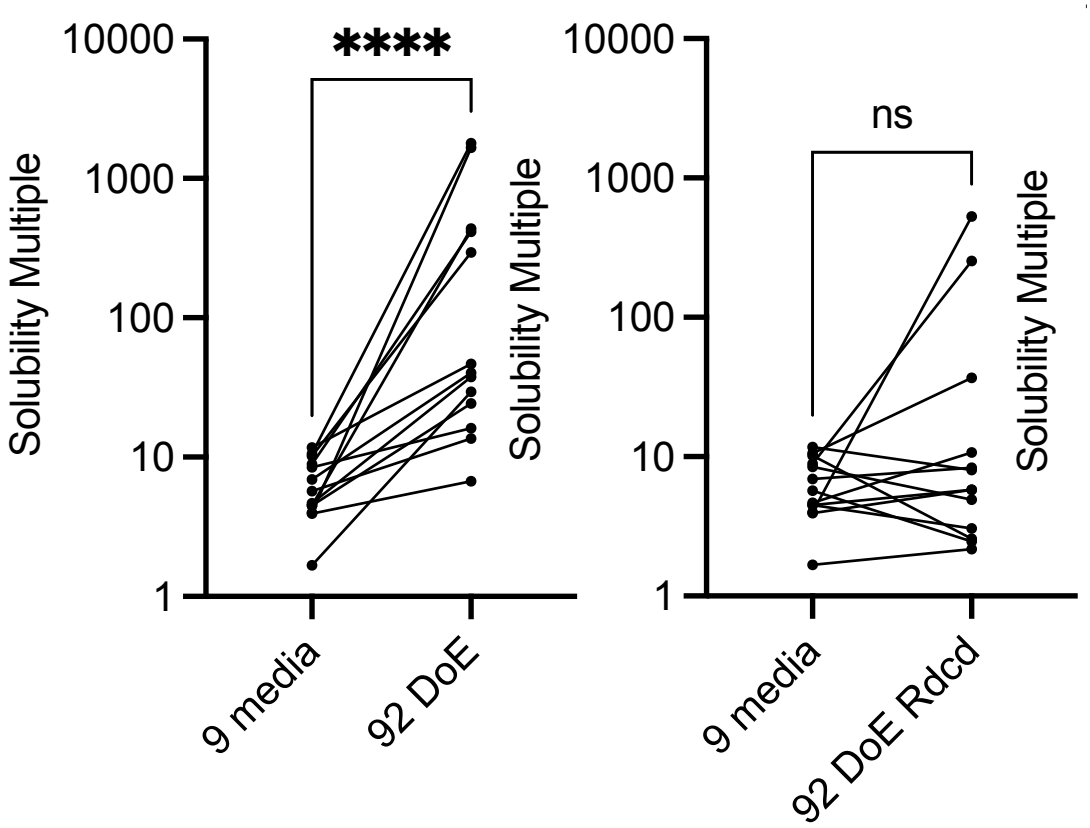


Figure 17. **Collected Solubility Multiple Comparison**

Note: 9 media this study, DoE 92 from [130], **** $p < 0.0001$; 92 DoE Rdcd this study (see text); ns = no significant difference.

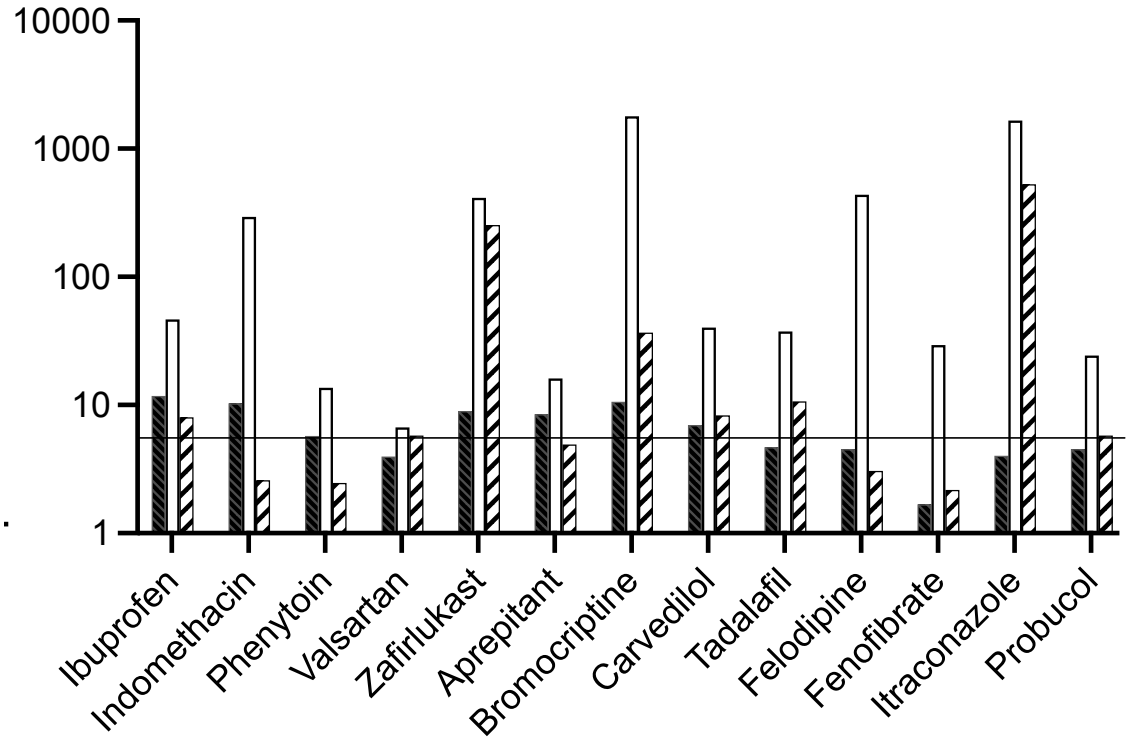


Figure 18. **Individual Solubility Multiple Results**

Note: ■ 9 media, this study; □ DoE 92 values from [130], ▨ DoE 92 Rdcd, see text; Horizontal line y = 5.71 phenytoin 9 media solubility multiple value.

3.3.3. Significant Factor Analysis

Although the 9 media composition is based on a multidimensional analysis of FeHIF [126] it is possible to fit the component values into a tailored DoE structure [123]. This permits a standardised effect value to be calculated for the impact of each media component on drug solubility but does not permit the calculation of two-way or higher interactions. The results are presented in Table 12, along with effect values from the previous equilibrium fed DoE studies [130-132]. This reveals that the 9 media system was not able to determine any significant standardised effect values occurring within the system. This is in contrast to the 92 point DoE study [130] where significant media components were identified for almost all drugs. The absence of detection is in agreement with previous results for the fasted 9 media system [127] where the number of significant factors decreases from the large scale DoE to the 9 media system. This also reflects the discussion in section 3.3.1, relating to the design of the media compositions within each system. The results indicate that if the number of data points is reduced, the data point compositions are not statistically driven, and

the solubility variability reduces, the experiment's ability to detect significant media components is severely impaired.

Table 12. **Significant Factor Analysis**

Fed Environment Significant Factors			
Drug	**9 media**	**9 DoE**	**92 Point DoE**
Indomethacin	NS	pH	pH, oleate, bile salt
Ibuprofen	NS	NS	pH
Phenytoin	NS	-	Bile salt, lecithin, pH, oleate
Fenofibrate	NS	Oleate	Oleate, bile salt, lecithin, buffer, monoglyceride
Felodipine	NS	Bile salt	Oleate, bile salt, pH, lecithin
Aprepitant	NS	NS	Oleate, bile salt, pH
Carvedilol	NS	NS	Bile salt, pH, buffer, oleate
Tadalafil	NS	NS	Bile salt, oleate
Zafirlukast	NS	NS	pH, bile salt, oleate
Probucol	NS	NS	Bile salt, monoglyceride, oleate, lecithin,pH
Valsartan	NS	NS	pH, Bile salt
Itraconazole	NS	NS	pH, oleate, bile salt, lecithin
Bromocriptine	NS	NS	NS
NS - No Significant Factors Found			

3.4. Conclusions

The 9 media approach using a small number of media recipes derived from a multidimensional analysis of sampled FeHIF [126] effectively measured a fed intestinal equilibrium solubility distribution. The equilibrium solubility measured with the 9 media system is only statistically equivalent to the initial fed 92 point DoE [130] in four out of thirteen cases (31%), but equivalent in sixteen out of twenty one cases (76%) to previous smaller DoE studies (DoE 10 [131] and DoE 9 [132]). The result can be related to the differences between the systems in media component concentration ranges, methods applied to determine media compositions and number of data points measured. The initial fed 92 point DoE [130] applies excessive media component concentration ranges compared to the 9 media system and elimination of outlier media

compositions improves the statistical agreement to 70%. This highlights that for SIF systems with similar media component concentration ranges and number of measured data points a statistically equivalent but not necessarily bioequivalent solubility space will exist. Due to the derivation of the 9 media system component concentrations and compositions, this system is more likely to represent the fed intestinal solubility range, present, within the limitations of the initial sampling study [159], than previous DoE approaches [130-132]. The comparison with the 92 point DoE indicates, that large scale DoE approaches generate statistically sensible but not biorelevant media compositions.

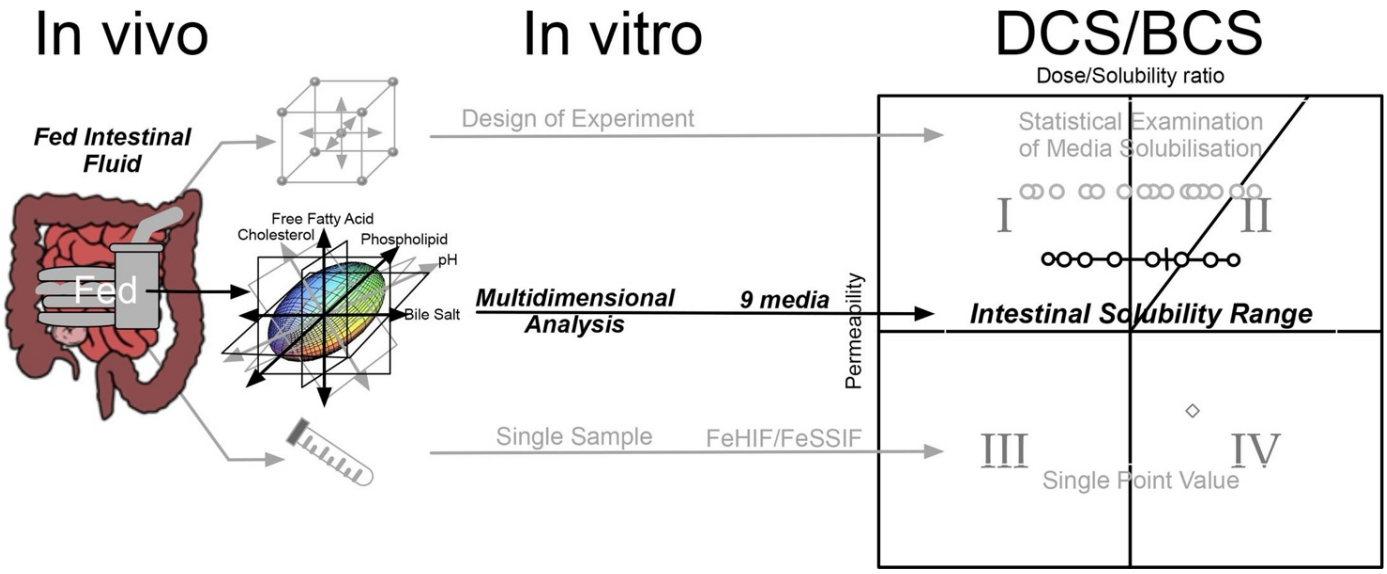
The solubility multiple (highest solubility / lowest solubility) for each drug observed using the 9 media system was smaller than the value from the initial fed 92 point DoE, a result due to the media differences discussed above and which indicates that the 9 media system probably provides a more realistic estimate of FeHIF solubility. Several drugs display very low solubility multiples, for phenytoin and tadalafil this is similar to their behaviour in the 9 media fasted system [127]. Indicating that in both fasted and fed states the intestinal solubility of these drugs is not sensitive to media composition. The neutral drugs also display very low solubility multiples, a new finding not present in the fasted 9 media system, which potentially impacts biopharmaceutical variability in the fed state in vivo and worthy of further investigation.

The fed 9 media system when analysed as a DoE does not detect any significant media factors influencing solubility. This arises due to the smaller number of media compositions tested, the derivation of the compositions and the lower solubility variability present in the fed state. This result is identical to the fasted systems and highlights that the DoE and multidimensional simulated intestinal media systems are exploring different solubility facets and appropriate choice will provide the required outcome.

The multidimensional fed 9 media system performs in a similar manner to the fasted version but also reveals different solubility behaviours. The system is worthy of further investigation using studies that relate the *in vitro* behaviour to in vivo performance.

Chapter 4

Fed Intestinal Solubility Limits and Distributions Applied to the Developability Classification System



This chapter includes the full text of the published article in European Journal of Pharmaceutics and Biopharmaceutics. (Silva, M. I., Khadra, I., Pyper, K., & Halbert, G. W. (2023). Fed intestinal solubility limits and distributions applied to the Developability Classification System. Eur J Pharm Biopharm, 186, 74-84. https://doi.org/10.1016/j.ejpb.2023.03.005) including the introduction, methods, results and conclusions.

For this work, I designed, analysed, and carried out all experiments and prepared the manuscript draft.

4.1. Introduction

4.1.1. Oral Drug Administration

The pharmaceutical industry favours oral administration as the most common route for drug delivery. The ease of ingestion and familiarity with this route are convenient and known to increase patient compliance and treatment effectiveness when compared with other delivery routes [164]. Notwithstanding these positive characteristics, there are challenges associated with the gastrointestinal tract that might be underestimated when it comes to the choice of this route. The gastrointestinal tract's anatomy and physiology, as well as the drug and medicinal product's physicochemical characteristics are factors that impact performance after oral administration [139]. To be absorbed from the gastrointestinal tract solid drug must first dissolve within the intestinal fluid and then permeate through the tract membranes to gain access to the portal and then systemic blood circulation. Therefore, intestinal solubility [165] along with permeability are two key factors controlling gastrointestinal drug absorption. Solubility and permeability are connected in the BCS [143] and the DCS [28], which link *in vitro* solubility and permeability to provide categorisations that predict a drug´s in vivo performance. Intestinal solubility is therefore a key parameter controlling oral absorption behaviour.

Drug solubility in simple aqueous buffers is not necessarily equivalent to intestinal solubility due to the influence of intestinal fluid components such as endogenous bile salt or free fatty acids from digested food [140]. The ultimate measure of intestinal solubility is using sampled HIF. However, it is known that the co-administration of drugs with or after food can significantly influence the rate and extent of drug absorption [166]. Intestinal fluid composition varies between fasted and fed states [159] and the two systems are usually investigated separately [149].

4.1.2. Fasted and Fed States

The gastrointestinal tract's normal physiological function is the digestion and absorption of food. A food effect occurs when a drug´s bioavailability significantly varies in the fed state when compared with the fasted state [167]. The fasted state is achieved by overnight fasting to ensure that the stomach and small intestine are devoid of food based materials. The sampled fasted intestinal fluid therefore represents a base level composition of gastrointestinal physiology in the

absence of exogenous food based materials [149]. The fed state is a more complex system arising after the ingestion of food resulting in a distinctive gastrointestinal fluid composition and volume, pH, surface tension, osmolality and variability associated with the nature of the food consumed [168]. Drug absorption in the fed state can therefore be influenced by the type of meal (solid or liquid), its calorie content, fluid ingestion, nutrient composition (high-fat meals, high-protein, or high-carbohydrate), volume and the temperature of the meal [168-171]. Fed state conditions are also associated with post prandial changes of the GI physiological variables for example bile flow, pH, different gastric emptying times and small intestinal transit times, changes in luminal metabolism along with direct food-drug interactions. All these factors can result in an increase (positive food effect) or decrease (negative food effect) in the overall extent of bioavailability [167, 171-173].

4.1.3. Human and Simulated Intestinal Fluid

A recent modification of the DCS [174] specified the preferred usage of HIF in order to provide improved standardised and biorelevant conditions for solubility determination. However, there are multiple practical issues that hamper HIF application in routine studies. The process of collecting HIF aspirates is complicated as it requires human volunteers and an invasive and variable technique [149]. Due to these limitations HIF from either fasted (FaHIF) or fed (FeHIF) states is expensive to obtain and inconsistent as it varies depending on different sampling protocols, storage conditions [140, 142], along with variability between different locations of the gastrointestinal tract and inter and intra subject variability [150, 159, 175].

To mitigate HIF collection and variability issues, SIF were developed and multiple recipes are available in the literature [155] covering both fasted (FaSIF) and fed (FeSIF) states. Drug solubility varies with SIF recipes [150, 176], which complicates the decision on which recipe is optimal [155]. The variability and complexity of fasted and fed SIF media systems was revealed in recent DoE [123, 130, 131, 160] that aimed to investigate the impact of SIF media components on drug solubility. These studies highlighted that intestinal solubility was a range and multiple media factors influenced solubility. To refine SIF recipes a subsequent publication [126] studied fasted and fed HIF sample compositions obtained from twenty volunteers [159] using a five dimensional (a dimension was either pH, bile salt, phospholipid, free fatty acid or cholesterol concentration) mathematical analysis. This identified for both the fasted and fed states eight media compositions that statistically

characterised over 95% of the HIF samples' component variation and calculated a centre point through a Euclidean approach. The nine fed SIF recipes have been utilised to determine the equilibrium solubility of a range of drugs previously studied in the fed DoE systems [177]. This study reported statistical equivalence to the previous small scale fed DoE studies [131, 132], along with the larger scale study [130] once solubility values from non-biorelevant media compositions were removed. In a similar manner to the fasted nine media system [127, 161] the fed version is more likely to represent the fed intestinal solubility range than the previous fed DoE studies [123, 130-132], with due recognition of the original HIF collection study's limitations [159].

4.1.4. Fed Developability Classification System and Solubility Driven Food Effects

The importance of studying solubility under physiologically relevant conditions was highlighted by Zaki N., et al [178] who demonstrated that some BCS Class II compounds when tested using relevant media (FaSSIF, FeSSIF and phosphate buffer pH 6.5) may perform differently in vivo and change their BCS Class. The authors emphasised that physiologically relevant conditions should be considered in all stages of drug discovery to produce better formulations. The published DCS analyses [28, 174] utilises solubility values for the fasted state but does not apply this to fed state. Since it is well known that solubility can vary between the fasted and fed state, the inclusion of fed solubility values would increase the information about a drug's behaviour in both states. This is especially important for poorly soluble drugs due to the potential for greater solubility changes in the fed state.

In this study, drugs originally tested in the fasted DCS [28] (furosemide, ibuprofen, mefenamic acid, paracetamol, acyclovir, griseofulvin and dipyridamole) were utilised to measure their equilibrium solubility in the fed intestinal fluid media compositions [126, 177]. The solubility range determined using these media recipes are more likely to be bioequivalent, in a similar manner to the fasted state [161], since they originated from sampled FeHIF [159]. It should be noted that there is a limitation since the fed state in the original study was obtained via the administration of the liquid feed Ensure Plus™. The aim of this study was to apply the fed state solubility range to the DCS grid and associated calculations, which to our knowledge is not available in the literature. To assess the solubility behaviour across the population a solubility frequency distribution was also determined. However, intra- and intersubject variability cannot be analysed using this approach

because the frequency distribution arises from the combined measured HIF samples of the twenty volunteers in the original study.

An obvious comparison would be the fed data measured in this paper against the previous fasted state study [161]. However, in order to limit paper size and focus discussion on the fed state DCS this manuscript will be restricted to a basic comparison of fed vs fasted results. A more detailed fasted vs fed comparison with a view to elucidating possible detection and quantification of solubility based food effects will be covered in a subsequent paper (Chapter 7).

4.2. Materials and Methods

4.2.1. Materials

Sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl) and formic acid were purchased from Merck Chemicals Ltd. Lecithin S PC (phosphatidylcholine from soybean "98%") was purchased from Lipoid®Germany. Chloroform was obtained from Rathburn Chemical® and FeSSIF-v2 media from Biorelevant.com Ltd. Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$) was from Fisher Scientific. The active pharmaceutical ingredients griseofulvin, furosemide, dipyridamole and acyclovir were purchased from Merck Chemicals Ltd. Ibuprofen was purchased from BSAF chemical company, paracetamol was obtained from Mallinckrodt Pharmaceuticals and mefenamic acid from Sigma Aldrich. The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

4.2.2. Methods

4.2.2.1. Stock media solutions for solubility experiments

As previously described in Section 2.3.

4.2.2.2. Fed Simulated Intestinal Fluid (FeSSIFv2)

As previously described in Section 2.3.

4.2.2.3. Equilibrium solubility measurement

As previously described in Section 2.4.

4.2.2.4. HPLC analysis

As previously described in Section 2.4.2.1.

4.2.2.5. Data analysis

Data analysis and comparison was conducted using Graphpad Prism 9 for MacOSX.

4.3. Results and Discussion

4.3.1. Equilibrium Solubility Measurements

The measured equilibrium solubility for the nine fed state media recipes and FeSSIF V2 are presented in Figure 19 along with where available, literature solubility FeSSIF or FeHIF data. The drugs analysed in this study have not been measured in previous fed DoE approaches [123, 130, 131] and thus a comparison with these data sets is not possible. The majority of the published FeHIF and FeSSIF solubility values (69%, 18 of 26 values) lie within the solubility range measured in this study, indicating that the solubility range is consistent with literature fed state solubility values. The level of agreement is comparable to the fasted state study where 8 out of 11 (73%) literature points were inside the fasted solubility range [161]. The variability observed between the literature points and this study could be due to different measurement protocols, different media compositions (for example pH) and that the fed state can be achieved using different meal types [149].

Although the study drugs have not been assessed in previous fed DoE studies the solubility behaviour is, based on the individual drug's physicochemical properties (Table 8), consistent with published DoE results [123, 130, 132] and the developing fasted state literature [127, 161, 179]. The acidic drugs exhibit a different solubility behaviour in this study in comparison to the fasted state, which can be connected to the different pH ranges between the two systems (fasted pH 6.64 – 8.04, fed pH 5.97 – 6.59) in relation to drug pKa values, see later sections. Three out of the seven drugs (acyclovir, griseofulvin and paracetamol) provide a narrow solubility range, which is also reported in the fasted state [127, 161, 179]. This further reinforces that for these drugs variation of media composition is not a major solubility influence and extends this finding into the fed state. Although this might have been expected since the media components utilised in this study are identical to the fasted study [161]. This low solubility range property is not restricted to a specific BCS/DCS class (paracetamol – Class I; griseofulvin – Class II; acyclovir – Class III) in the fed state and is probably due to a combination of the drug's molecular structure and physicochemical properties. These compounds, when compared to the others in this study, are relatively simple planar molecules with a low log P value (Table 8, albeit griseofulvin log P = 2). In order to completely define this behaviour an increased number of examples would be required. These results highlight for the fed state that an *in vitro* multi point solubility analysis allows for the detection and study of properties and behaviours that would not be possible using a single point measurement [127, 161, 179].

To compare the fed nine media system with the FeSSIFv2 solubility values, a statistical comparison of the nine media centre point solubility value and the mean FeSSIFv2 solubility was performed using a Wilcoxon matched pairs signed rank test. This analysis indicates that there is no statistically significant difference between the two data sets (Figure 20) which suggests that the existing FeSSIF v2 could be compared with the centre point solubility measured with the fed media system. A non-parametric statistical comparison (Mann-Whitney test) of FeSSIFv2 and centre point measurements (n = 3 per drug for both systems) performed for each individual drug did not detect a significant difference. This statistical analysis is however, hampered by the small number of drugs tested and the limitations of a non-parametric test. A larger number of drugs or multiple measurements for individual drugs is required to fully confirm the results of this comparison.

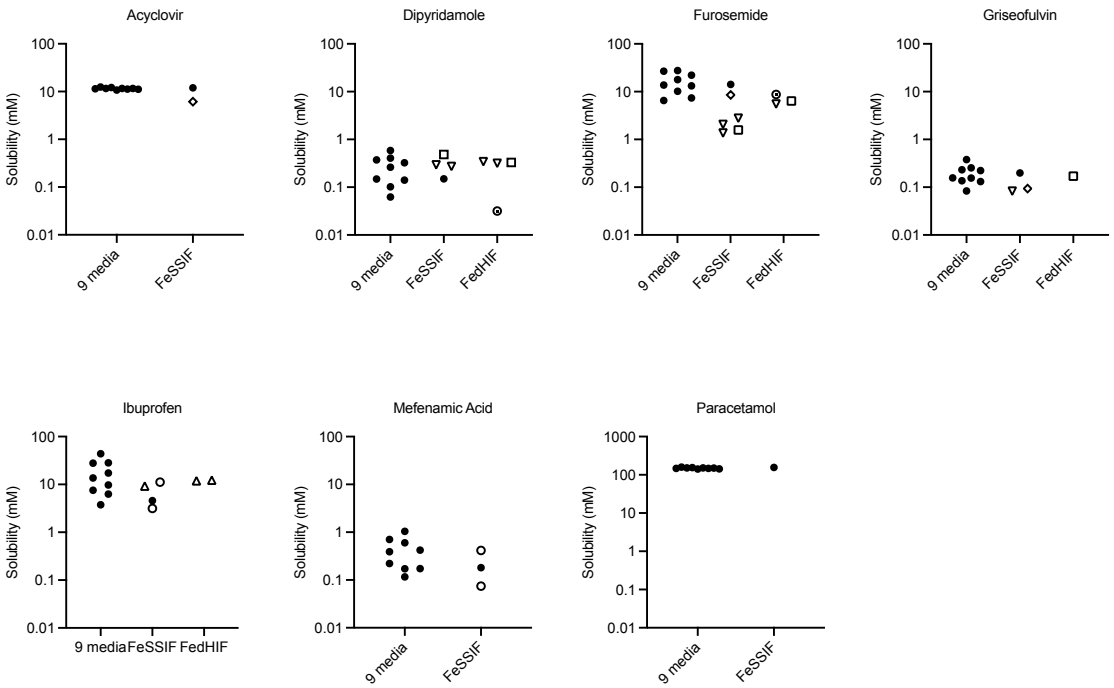


Figure 19. **Measured Fed State Equilibrium Solubility Distributions**

Note: ● 9 media this study (mean, n = 3); ● FeSSIFv2 (Fed Simulated Intestinal Fluid v2) this study (mean n = 3); FeSSIF (Fed Simulated Intestinal Fluid v1) and HIF (Fed Human Intestinal Fluid) literature values as follows △ from [142]; ◇ from [180]; □ from [150]; ◉ from [181]; ○ from [182]; ▽ from [151]. NB Paracetamol y-axis different scale.

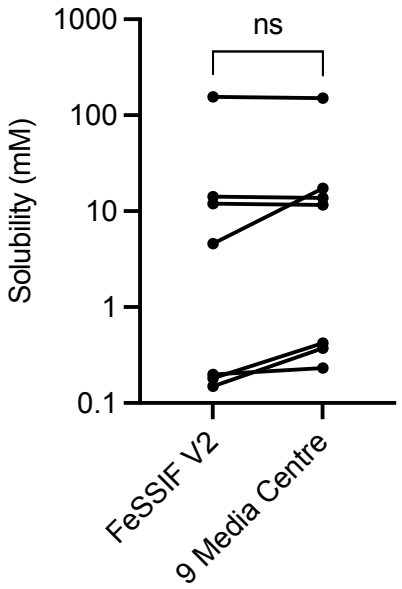


Figure 20. **FeSSIFv2 vs Centre Point Solubility Comparison**

Note: FeSSIFv2 (Fed State Simulated Intestinal Fluid) and Centre this study. ns no significant difference (P > 0.05), each point mean n = 3.

4.3.2. Solubility Range

Collected solubility data is presented in Table 13 along with the dose and Peff values from the original DCS publication [28]. The solubility multiplier was calculated using the maximum and minimum solubility values and ranges from 1.16 for acyclovir to 11.7 for ibuprofen. A skew value was also determined to assess distribution symmetry around the centre point media. A value of 1 indicates a broadly symmetrical distribution, values >1 indicate a skew to higher solubility values and conversely <1 to low solubility values. The calculated values range from 0.694 for dipyridamole to 2.04 for mefenamic acid. The solubility multiplier values are smaller than the original fed DoE [130]. A previous examination of the nine media fed system concluded that this was due to the elimination of non-biorelevant outlier media systems that resulted from the DoE statistical design [177]. Along with greater media concentration variation due to the upper and lower DoE limits. A comparison with the fasted nine media study (Table 13) [161] indicates some differences in multiplier values especially for the acidic drugs. Both mefenamic acid and furosemide exhibit a decreased solubility multiplier in the fed study in contrast with ibuprofen that shows an increase. This variation can be explained by the differences in media pH (fed pH 5.97 – 6.59 Δ = 0.62 / fasted pH 6.64 – 8.04 Δ = 1.4) in combination with the drugs' pKa values (see Table 8). This result is consistent with the previous finding that pH is the main factor controlling acidic drug solubility [130, 160]. Based on the solubility multiplier furosemide in the fed state has a narrow solubility range (similar to griseofulvin). In general, drugs with the lowest solubility multiplier also have the lowest skew value a result in agreement with the fasted study. However, dipyridamole is an exception in this fed study and presents the lowest skew value with a larger solubility multiplier, which may be an example of a complex drug behaviour in the fed state. Individualistic drug behaviours in these media systems has been previously reported [157, 158], however a larger number of data points is required to fully explore this behaviour.

Table 13. **Collected Solubility Data and Analysis**

Drug	**Dose** (mg)*	**Estimated Human Peff** ($cms^{-1}x10^{-4}$)*	**FeSSIF V2 Solubility** (mg/ml)	**Centre Point Solubility** (mg/ml)	**Minimum Solubility** (mg/ml)	**Maximum Solubility** (mg/ml)	**Solubility Multiplier**[1]	**Skew**[2]
Ibuprofen	400	12	0.946	3.58	0.773	9.06	11.7 (4.41)	1.96 (0.772)
Mefenamic Acid	250	14	0.044	0.102	0.028	0.252	9.02 (35.9)	2.04 (29.2)
Furosemide	80	0.6	4.68	4.56	2.17	9.13	4.21 (40.0)	1.91 (3.16)
Dipyridamole	100	1.5	0.076	0.188	0.031	0.297	9.44 (7.48)	0.694 (7.23)
Paracetamol	500	1.3	23.6	22.8	21.4	24.2	1.13 (1.22)	0.919 (1.10)
Griseofulvin	500	8.7	0.070	0.082	0.030	0.133	4.52 (2.32)	0.998 (3.63)
Acyclovir	800	0.25	2.70	2.61	2.42	2.81	1.16 (1.15)	1.059 (0.929)

* Data from Butler [28].

1: Solubility Multiplier = (Maximum Solubility)/(Minimum Solubility).

2: Skew = ((Maximum Solubility − Centre Point Solubility))/((Centre Point Solubility − Minimum Solubility)).

3: Solubility Multiplier and Skew, bracketed values from fasted study [161].

4.3.3. Developability Classification System Analysis

The DCS [28, 174] was developed to cover the fasted state and this is the first investigation of fed solubility data using this approach. The fed nine media solubility data was combined with the drug's normal oral dosage in the original DCS paper [28], to calculate a dose/solubility ratio for each media measurement and plotted at the respective permeability value. The results are presented in Figure 21, where it is possible to visualise the drug´s fed DCS dose/solubility range. The fed nine media compositions were designed to cover greater than ninety five percent of the intestinal fluid variation within a data set of fed HIF samples [126] (collected from twenty healthy volunteers [159]). Therefore it is reasonable to assume that the measured dose/solubility ratio ranges represent a drug´s solubility behaviour in fed intestinal space. As detailed previously there is a caveat to this assumption, the fed state in the original study [159] was obtained using 400mL of Ensure Plus as a liquid meal representative of a standard meal. This may not be equivalent to alternative fed states induced by solid meals [149]. The lowest solubility or largest dose/solubility ratio could be interpreted as the worst scenario in a drug´s solubility profile during the fed state with more than ninety percent of the dose/solubility distribution lower than this value. Thus, formulation selection and compound screening based on the lowest solubility values could be applied to early drug development as a worst case scenario instead of centre point or FeSSIF values. This could facilitate quality-by-design development approaches and reduce the risk of unexpected solubility induced behaviour. The knowledge of lowest solubility values for the fed state could also be especially useful since it might be able to highlight the impact of food effects on solubility, when compared to the fasted state.

For the acidic drugs, mefenamic acid, furosemide and ibuprofen, the solubility behaviour is highlighted with respect of media pH in Figure 22. The main conclusion is that solubility increases (thus dose/solubility volume decreases) with increasing pH, with some variations occurring due to media amphiphilic factors. As above this is consistent with pH as the major solubility driver for acidic drugs as identified in the original fed DoE study [130] and subsequent studies [131, 132]. Also comparable with the fasted system [161], indicating that acidic drug solubility behaviour remains consistent with the fed media.

An interesting biopharmaceutical result is a drug's position within the DCS. Figure 21 indicates that three drugs (paracetamol – Class I, furosemide and acyclovir – Class III) are within class

boundaries, with four drugs (ibuprofen Class I – II, mefenamic acid and griseofulvin Class IIa – IIb, dipyridamole Class I - IIa – IIb) spanning across boundaries. This is markedly different to the fasted nine media result where only mefenamic acid crossed a classification boundary [161]. In the fed state mefenamic acid crosses a DCS boundary, from IIb (solubility limited) to IIa (dissolution limited) with the centre point and FeSSIFv2 values both located in IIa and only a single lowest solubility value located in IIb. In the fasted state mefenamic acid also crossed between IIa and IIb, but the centre point was located on the class boundary. Similarly, for ibuprofen a single low solubility value crosses from Class I into IIa. Griseofulvin is similar crossing from IIa to IIb, but in this instance only the single highest solubility value lies within IIa. This is also different to the fasted state where all points are located within Class IIb. This shift to a higher solubility and lower dose/solubility ratio in the fed state correlates with the known effect of food enhancing griseofulvin bioavailability [183]. Dipyridamole, has a Peff value that is very close to the low/high permeability boundary and in the fed nine media system is the only drug to cross two classification boundaries, spanning from two high solubility values in Class I, three in Class IIa and four (along with FeSSIFv2) in Class IIb. This behaviour is different to the fasted state where all the measured solubility values were in Class IIb. This additional information regarding the variations on drug solubility behaviour in the DCS grid is only available due to the solubility range that results from the multipoint measurements. The same analysis would not be possible with single measurements using FeHIF or FeSIF, and is a further example of the utility of this solubility range approach.

A DCS grid including all 23 drugs studied in this thesis is available in Appendix A.

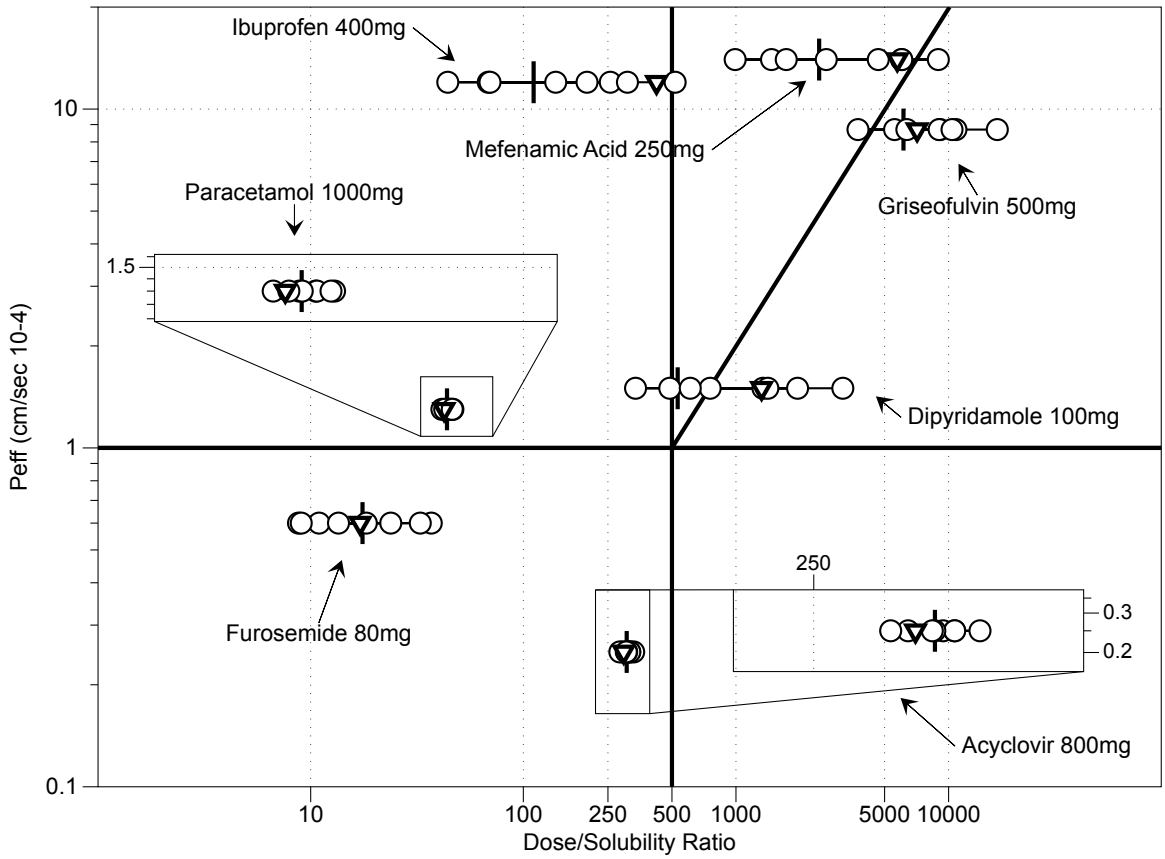


Figure 21. **Fed Nine Media Systems on Developability Classification System Grid**

Note: ▽ FeSSIFv2 (Fed State Simulated Intestinal Fluid); ○ Fed Nine Media data points, I Fed Nine Media centre point. Inset expanded scale for acyclovir and paracetamol. Individual drugs and doses as labelled. Each point mean n = 3.

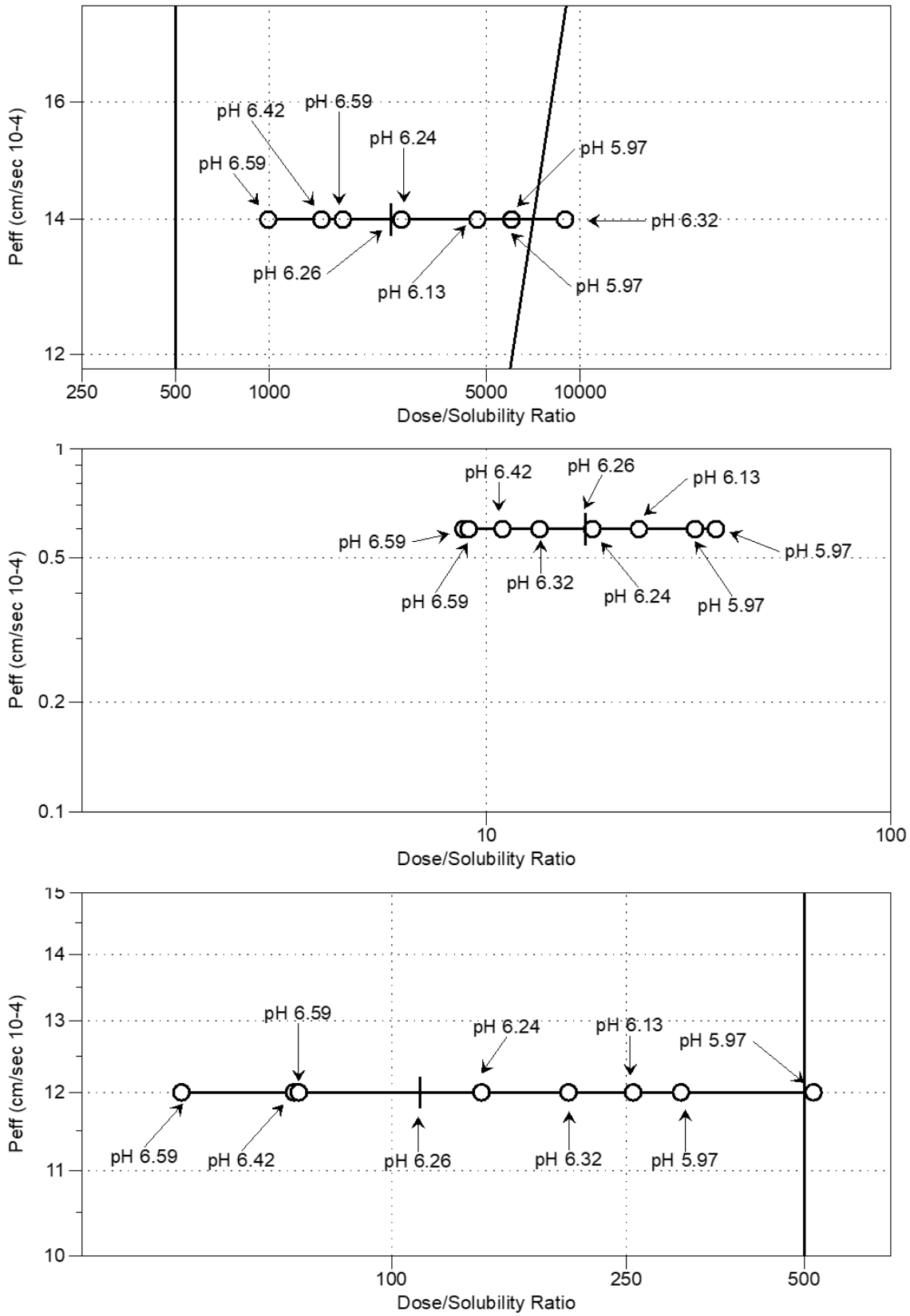


Figure 22. **Acidic Drugs pH driven solubility behaviour**

Note: (a) Mefenamic Acid. (b) Ibuprofen. (c) Furosemide. ○ Fed Nine Media data points, I Fed Nine Media centre point. Media pH values as labelled, see Table 7. Each point mean n = 3.

4.3.4. Fed Solubility Distributions

Table 7 media compositions were calculated based on the compositional variation of the 172 fed HIF samples in the original analysed data set [126]. Through the application of 5-dimensional Euclidean space it is possible to calculate the proximity of each fed HIF sample to an individual media composition (Table 7) to produce a frequency distribution based on the number of HIF samples closest to each media. The equilibrium solubility of each media can then be converted to a dose/solubility volume vs frequency distribution, see Figure 23a and Figure 23b. It should be noted that this frequency distribution arises from the sampled fed HIF point compositions [126, 159] and cannot be related to individual subject in vivo pharmacokinetic variability [184].

In Figure 23a the distributions for paracetamol, acyclovir, griseofulvin and dipyridamole are presented. Based on the presentation in Figure 1 and associated discussion in section 4.3.1, paracetamol, acyclovir and, griseofulvin all have very narrow frequency distributions with almost vertical cumulative lines, related to the very narrow solubility range for these drugs. Dipyridamole has a broader distribution range but the points are not evenly distributed on the cumulative plot and the centre point is towards the higher end of the plot. In Figure 23b the distributions for mefenamic acid, ibuprofen and furosemide are presented. Since these are all acidic drugs the distributions will be predominantly controlled by pH (see section 4.3.2 and Figure 22), but also display the same characteristics previously described. Mefenamic acid and ibuprofen also exhibit an increased degree of structure in the cumulative plot with steps in the distribution.

Statistical analysis of the distributions either for normal or log normal behaviour did not produce significant results. Previous statistical analysis of fed SIF DoE solubility distributions [123, 131] highlighted that the distributions were not normal, also the fed HIF data points used to calculate the bioequivalent points [126] were not normally distributed. This result might reflect the well known variability of these fluids [185, 186] and the measurement of solubility in them [147, 158, 187]. This behaviour is similar to the fasted system [161] and a comparable analysis highlights that the change from low to high solubility is not a simple vector based on the increasing concentration of a single media component. Therefore, the lack of an organised statistical distribution when traversing the solubility range based on individual discrete points is to be expected. This highlights why a single fed HIF aspirate will not be representative of the entire fed HIF space and single measurements limited by a lack of knowledge of the sample's position in the space, which will be further complicated when drug properties are superimposed.

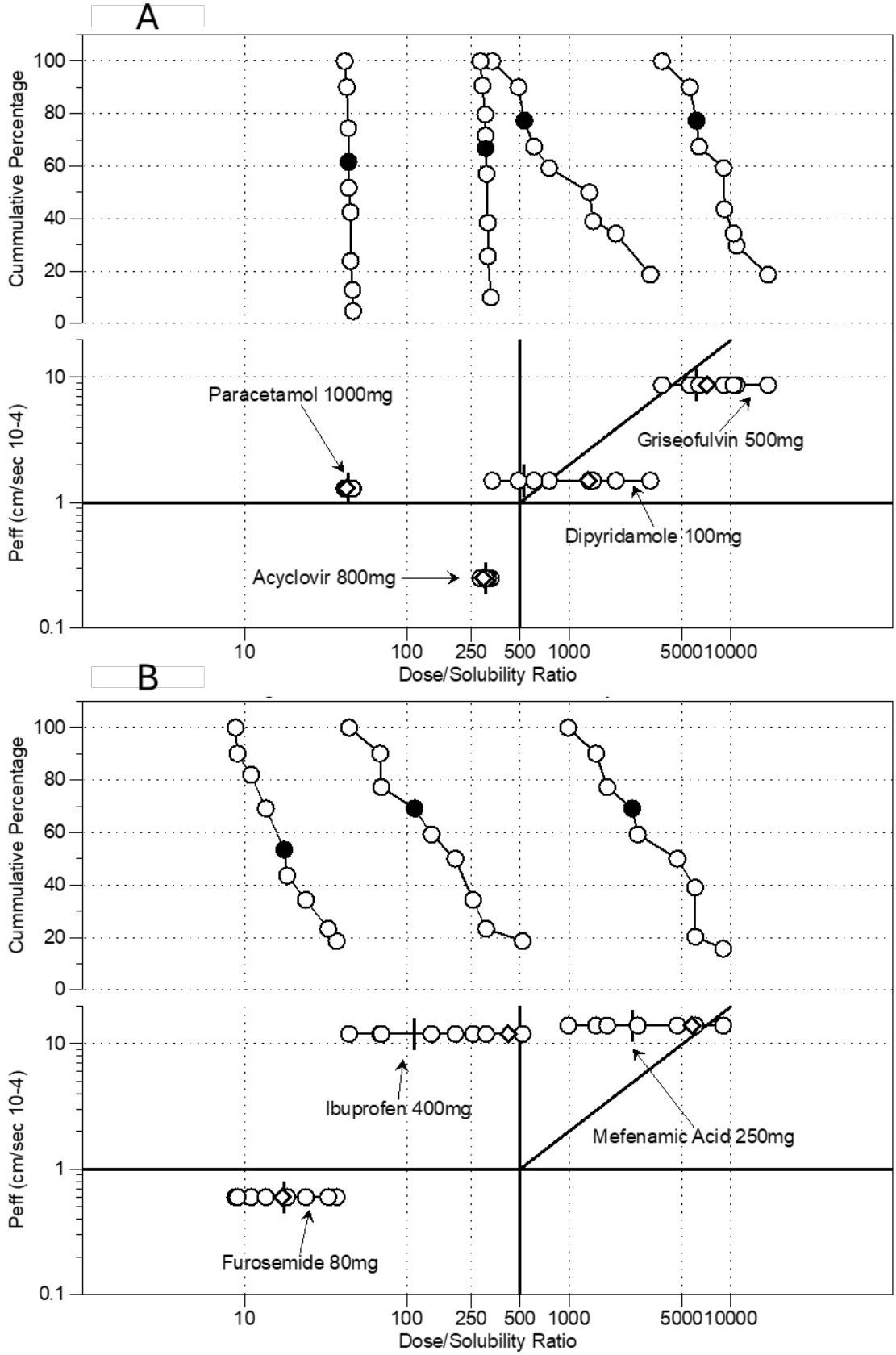


Figure 23. **Cumulative fed dose/solubility ratio distributions**

(a) Upper graph: Cumulative percentage incidence of data points, O Fed Nine Media data points, ● Fed Nine Media data centre point. (b) Lower graph: Developability Classification System Grid, ◊ FeSSIFv2 (Fed State Simulated Intestinal Fluid); O Fed Nine Media data points, I Fed Nine Media data centre point. Each point mean n = 3.

4.3.5. Solubility Limited Absorbable Dose Distribution

A SLAD and target particle size to avoid dissolution rate-limiting issues can be determined by applying biopharmaceutical assumptions and calculations [28, 174]. The SLAD calculation requires a value for the total volume of intestinal fluid. This has been determined as 1150 mL based on 500 mL for a fasted system [28], plus the volume administered during the fed phase of the intestinal fluid sampling study [159] 400mL Ensure Plus + 250 mL water. This increase in fed state volume in comparison to the fasted means that the calculated fed SLAD will be 2.3 times higher than the fasted value even if the drug's measured solubility does not change. This calculation has been applied to the centre point and lowest solubility value as a worst case situation (Table 14), using literature Peff values [28] and standard values for other properties.

A comparison of the calculated values for the centre point and lowest solubility measurements not surprisingly exhibit the same relationship described above for solubility. For narrow solubility distribution drugs (paracetamol, acyclovir, griseofulvin and furosemide) there is minimal difference between the values, whilst for the other drugs the difference reflects the discussion above. For paracetamol, acyclovir and griseofulvin this finding matches the fasted state (Table 14, bracketed values). This indicates that a narrow intestinal solubility range might be a useful drug development target, since the drug would be intrinsically resistant to intestinal solubility variability. It could also be surmised that congruent fasted and fed solubility distributions would further enhance resistance to gastrointestinal food effect solubility issues. The narrow distribution for furosemide is only present in the fed state, this represents a different behaviour to the fasted and linked to the lower fed media pH range and drug pKa, see above. This indicates that for furosemide, population plasma concentration variation in the fed state should be lower than the fasted, assuming solubility controlled absorption and no interference from other factors, metabolism for example. In one study the AUC in the fasted state is 2,174 ± 668 ng/ml.h and 1,219 ± 403 ng/ml.h in the fed state [188], whilst a separate study determined that the fasted area was 51.3 ± 7.24% (with reference to an IV dose) and the fed 43.3 ± 5.94% [189]. In both cases the fed variability is lower, possibly due to the solubility effect noted, with one study [189] stating, "food seemed to diminish the interindividual differences". Although not conclusive, due to the variations in the studies (Beermann determines that there is a food effect on bioavailability, whilst Hammarlund does not find a food effect), this result indicates the potential utility of comparing the fasted and fed solubility distributions as an

indicator of food effects. For four drugs (ibuprofen, mefenamic acid, furosemide, paracetamol), the calculated lowest SLAD is above the administered dose (Table 13 and Table 14), which indicates that minimal solubility based absorption issues are possible and reflective of their positions on the DCS grid. For three drugs (dipyridamole, acyclovir and griseofulvin), the calculated lowest SLAD is below the administered dose (Table 13 and 14, acyclovir also the centre point value) and therefore the lowest solubility based calculation could be applied as a quality-by-design parameter for particle size to reduce the risk of absorption issues [174]. By linking a point's SLAD value to the cumulative percentage incidence (see section 4.3.4), it is possible to determine where solubility limitations no longer apply. This is presented in Figure 24 for dipyridamole, acyclovir and griseofulvin. For dipyridamole and griseofulvin the plot indicates that solubility limitations will arise in under forty and sixty percent of fed HIF compositions respectively and this information could be applied for a risk assessment based development and formulation. For acyclovir all SLAD points are lower than the dose, however the difference is approximately 100mg or 12% of the dose, which may not be critical in vivo.

There are interesting differences between the fasted and fed SLAD analysis for these drugs. For griseofulvin all fasted SLAD values were below the dose, see Figure 24 [161], whilst in the fed state 60% of the population is below the dose. For dipyridamole a similar situation exists, in the fed state with a shift to only 40% of the population below the administered dose. In the fed state all acyclovir SLAD values are below the administered dose, but this is reversed in the fasted state. Whilst in the fasted state 65% of mefenamic acid is below the SLAD but no points are in the fed state. As discussed above for griseofulvin this reflects the well known impact of food on bioavailability [183] and food is also known to increase the bioavailability of dipyridamole [190, 191]. The literature for acyclovir indicates that it does not exhibit food effects [192]. However, it is a low permeability (Class III), low bioavailability (0.15-0.2) drug and the change in solubility noted in this study might not be sufficient to provide a detectable effect in vivo. Overall the comparison of the fed solubility profile determined in this paper with the previous fasted determination is highlighting differences *in vitro* between the two states that potentially represents the impact of food in vivo on gastrointestinal solubility.

Table 14. **Calculated Biopharmaceutical Data**

Drug	SLAD[1] (mg)		Particle Radius (µm)	
	Centre Point Solubility	*Minimum Solubility*	*Centre Point Solubility*	*Minimum Solubility*
Ibuprofen	47,205 (24,519)	10,199 (8,380)	231	107
Mefenamic Acid	1,539 (193)	431 (90)	39	20
Furosemide	3,009 (1,181)	1430 (114)	261	179
Dipyridamole	310 (10)	52 (6)	53	22
Paracetamol	32,669 (12,357)	30652 (11,183)	584	566
Griseofulvin	780 (55)	282 (43)	35	21
Acyclovir	718 (3,434)	695 (3,186)	198	194

Solubility Limited Absorbable Dose - SLAD = S_{INT} x V x A_n where S_{INT} is the intestinal solubility (mg/ml) measurement as indicated in column header (see Table 9), V is the volume of fed intestinal fluid (1150 ml) and A_n is the absorption number ($A_n = \frac{P_{eff} \, x \, T_{si}}{R}$) where P_{eff} is the effective permeability of the intestine to the drug (see Table 13), T_{si} is the small intestinal transit time (3.32 hours) and R is the intestinal radius (1.25 cm). Note V value based on 500 ml of fasted system [28], plus volume administered during fed phase of intestinal fluid sampling study [159] 400ml Ensure Plus + 250 ml water.

Particle radius = $\sqrt{3D \, x \, S_{INT} \, x \, T_{si} / D_n \, x \, \rho}$ where D is the diffusion coefficient (typically at 5 x 10^{-6} cms^{-1}), S_{INT} and T_{si} are as above, D_n is the dissolution number (set to 1) and ρ is the drug density (typically 1.2 g cm^{-3}).

1: SLAD, bracketed values from fasted study [161].

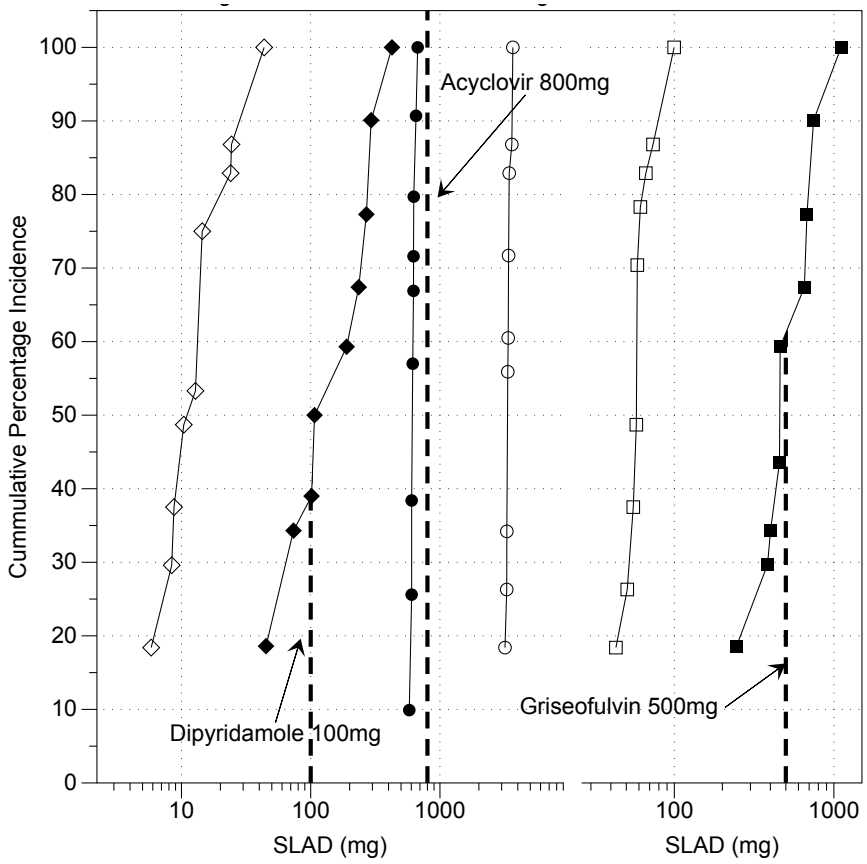


Figure 24. **Cumulative Percentage Incidence of Solubility Limited Absorbable Dose**

Note: Closed symbols Fed Data, Open symbols fasted data from [31]. ◊ Dipyridamole, ○ Acyclovir, □ Griseofulvin, dotted vertical line drug dose, value as indicated.

4.4. Conclusions

These results indicate that the nine fed media recipes are simple to apply and provide drug equilibrium solubility measurements in agreement with literature fed HIF and SIF values and solubility behaviour in agreement with previous DoE studies. The solubility values can be applied to calculate fed dose/solubility points that can be plotted on the DCS grid, and due to the derivation of the nine fed media recipe compositions are likely to cover greater than 95% of the fed intestinal solubility range. Application of standard oral biopharmaceutical parameters also permits the calculation of a SLAD value, which further enhances the available information. The range provides greater information than single point measurements and the lowest solubility value represents a worst case scenario that could be applied to risk assessment or quality-by-design approaches during drug screening, development and formulation. Solubility values can be linked to the original HIF

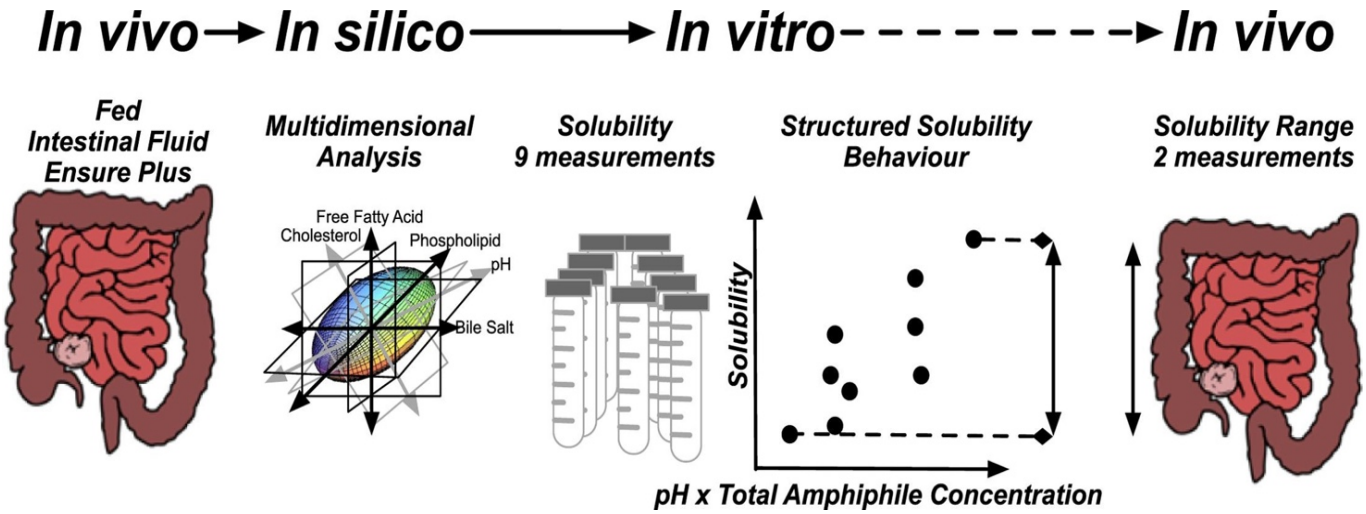
data set to provide a population frequency distribution that further refines the risk assessment. This approach is comparable to the nine fasted media recipe system [161].

A comparison of the fed values in this study with the fasted values from a previous study [161] reveals some interesting differences in solubility behaviour for griseofulvin, dipyridamole, furosemide and acyclovir. These *in vitro* fasted vs fed differences can be reconciled with the results from in vivo studies that have examined the impact of food on oral absorption. This indicates that the combination and comparison of the fasted and fed solubility ranges *in vitro* might be a useful indicator of in vivo behaviour. This will be explored further in a subsequent paper.

Overall the approach is therefore worthy of further development and research to expand the number of drugs analysed, link *in vitro* solubility to in vivo pharmacokinetics and investigate the fasted fed state comparison.

Chapter 5

Structured solubility behaviour in fed simulated intestinal fluids



This chapter includes the full text of the published article in European Journal of Pharmaceutics and Biopharmaceutics. (Silva, M. I., Khadra, I., Pyper, K., & Halbert, G. W. (2023). Structured solubility behaviour in fed simulated intestinal fluids. Eur J Pharm Biopharm. https://doi.org/10.1016/j.ejpb.2023.10.017) including the introduction, methods, results and conclusions.

For this work, I designed, analysed, and carried out all experiments and prepared the manuscript draft.

5.1. Introduction

The most popular choice to administer medication is through the oral route, which enables patients to self-medicate and enhances patient compliance and tolerance of treatment [139]. For the pharmaceutical industry, this route has advantages since it allows the preparation of stable solid formulations that are cost effective. However, to achieve systemic therapeutic effects the drugs in oral formulations need to be absorbed from the gastrointestinal tract and enter the bloodstream [140, 193]. Dissolution is therefore a crucial step in oral administration that can be influenced by the drug's physicochemical properties, formulation, gastrointestinal tract physiology and patient's food intake and clinical condition [193]. Since drugs cannot be absorbed in their solid form, dissolution is a vital step and solubility is known to play a significant role in this process [194]. The importance of solubility was highlighted in the BCS [143] and further refined in the DCS [28, 174, 195] where intestinal solubility and permeability were linked to in vivo absorption.

Administering drugs with poor solubility may lead to incomplete and inconsistent drug absorption therefore, measuring in vivo intestinal solubility *in vitro* is a key stage in drug development [147, 196]. Drug related factors such as pKa, logP, chemical structure and gastrointestinal factors such as tract physiology and anatomy along with patient related factors such as age, lifestyle and disease state, can affect intestinal solubility [163, 196]. Therefore, simple aqueous and buffer solubility approaches may not always reflect the gastrointestinal solubility. To address this issue, two options are available. One involves measuring solubility in HIF samples [150, 197, 198]. The other uses SIF [116, 146, 147] to assess intestinal solubility *in vitro* and to simulate either the fasted (FaSSIF) or fed (FeSSIF) states.

When FeSSIF were introduced [84], the aim was to simulate critical aspects of the gastrointestinal environment that were not considered when measuring solubility in aqueous buffer systems. The recipes were based on available HIF composition data and included important elements such as bile salts, lecithin and pH [86, 153]. More complex recipes with free fatty acid, monoglyceride and enzyme components (FeSSIF-V2) were also developed and intended to help understand the intricate interactions of drugs in the GI tract especially after food consumption. Several *in vitro* - in vivo correlations are available [142, 150, 175, 196], however, different FeSSIF recipes are applied without a consensus on which is optimal [199]. The fed conditions also present specific challenges that can hamper a comparison between studies. The type of meal (solid or liquid), its composition and calorie content, the amount of fluid ingested and the collection

technique are among the factors that can affect comparisons [168, 171]. The fed state is associated with large HIF variability that is not simulated with single FeSSIF media and approaches where only one solubility value is determined are not covering the full in vivo fed solubility range.

In recent statistical DoE multiple combinations and concentrations (high and low) of FeHIF media components[1] were tested in order to study the solubility variability [123, 130-132]. These approaches are great tools to study the key media components affecting solubility and the complex interactions between them, highlighting that intestinal solubility is a range. Although useful, their application to early drug development is limited by the heavy experimental resource required (the published fed DoE required 92 experiments per drug [130]) and their statistically constructed media recipes may not be biologically relevant. A subsequent study performed a multidimensional mathematical analysis of fasted and fed HIF composition [126] (pH, bile salts, phospholipid, free fatty acid, and cholesterol) obtained from twenty human volunteers [159]. This analysis resulted in eight media recipes for the fasted and fed states that statistically characterised over 95% of the HIF samples' component variation plus a calculated centre point through a Euclidean approach. This approach potentially generates solubility data with improved bioequivalence using fewer experiments and could be an alternative to current FeSSIF media for biopharmaceutical studies.

A recent paper [200] compared the equilibrium solubility in fed simulated intestinal media systems of a group of 13 drugs (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast, aprepitant, carvedilol, tadalafil, bromocriptine, fenofibrate, felodipine, probucol, itraconazole) using two approaches, either a multidimensional analysis [126] (9 media system) or DoE (92DoE [130], 10DoE [131], 9DoE [131]). Statistical differences between the data sets highlighted that larger scale DoE (92DoE) approaches generate FeSSIF compositions with excessive component concentration ranges and combinations not likely to be equivalent to FeHIF. The 9 media system recipes, which are derived from FeHIF compositions, are more likely to represent fed intestinal media than statistical DoE approaches and therefore could be considered to provide a bioequivalent solubility measurement. It should be noted that there is a limitation since the fed state in the original study [159] used to derive the fed 9 media system was obtained via the administration of the liquid feed Ensure Plus™ which is not equivalent to solid meals.

The equilibrium solubility of a further group of drugs (furosemide, dipyridamole, mefenamic acid, ibuprofen, griseofulvin, acyclovir and paracetamol) was measured using the multidimensional 9 media system (Table 7) and applied to the original DCS grid [201]. The inclusion of nine fed

intestinal solubility values instead of the traditional single measurement approach (eg FeSSIF value) resulted in more information regarding the solubility behaviour of drugs, including the lowest solubility value that represents the worst case solubility scenario. This could be applied to risk assessment or Quality-by-Design (QbD) approaches in early development and formulation.

In this paper we have measured the equilibrium solubility of additional drugs piroxicam, carbamazepine, atazanavir and posaconazole (see Table 8). In combination with the equilibrium solubility values from previous studies (indomethacin, ibuprofen, phenytoin, valsartan, zafirlukast, aprepitant, carvedilol, tadalafil, bromocriptine, fenofibrate, felodipine, probucol, itraconazole) [200] (furosemide, dipyridamole, mefenamic acid, ibuprofen, griseofulvin, acyclovir and paracetamol)[201] our aim is to examine the solubility behaviour and determine patterns that can be applied to define drug categories. If present this would permit a reduction in the number of simulated intestinal media measurements required to establish a fed state solubility range. The determination of an *in vitro* maximum and minimum solubility would provide additional solubility information with less resource and could be applied in early drug development when API material is limited.

5.2. Materials and Methods

5.2.1. Materials

Merck Chemicals Ltd supplied sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl), and formic acid. Lipoid® Germany supplied Lecithin S PC, which is phosphatidylcholine derived from Soybean with a purity of 98%. Rathburn Chemical® supplied chloroform, and Biorelevant.com Ltd supplied FeSSIF-v2 media. Fisher Scientific provided sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$). The active pharmaceutical ingredients carvedilol, tadalafil, valsartan, piroxicam, naproxen, griseofulvin, fenofibrate, bromocriptine, phenytoin, itraconazole, indomethacin, probucol, ibuprofen, furosemide, dipyridamole, carbamazepine, and acyclovir were purchased from Merck Chemicals Ltd. Aprepitant and felodipine were provided through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark and zafirlukast was purchased from Stratech Scientific Ltd. Paracetamol was obtained from Mallinckrodt Pharmaceuticals and mefenamic acid from Sigma Aldrich. Posaconazole and atazanavir were purchase from ChemShuttle. All active pharmaceutical

ingredients were > 98% pure based on certificates of analysis. The physicochemical properties of the drugs in this study are displayed in Table 8. The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

5.2.2. Methods

5.2.2.1. Stock media solutions for fed solubility experiments

As previously described in Section 2.3

5.2.2.2. Equilibrium solubility measurement

As previously described in Section 2.4

5.2.2.3. HPLC Analysis

As previously described in Section 2.4.2.1

5.2.2.4. Data analysis

Data analysis and comparison was conducted using GraphPad Prism 9 and DataGraph 5.0 for MacOSX.

5.3. Results and Discussion

5.3.1. Solubility Analysis

The 9 fed state simulated media recipes applied in this paper resulted from a multidimensional analysis of five FeHIF components (bile salts, cholesterol, lecithin, free fatty acid and pH). The impact of these components on solubility can be studied in combination using a DoE approach [123, 130, 132] or as the sum of all component concentrations [202] (TAC, total amphiphile concentration in

mM). In this paper, to present the solubility data on an x-y coordinate system, each fed media recipe was simplified to a single value. This is achieved by either calculating the product of the total amphiphile concentration and media pH (Table 7) or using pH alone. A published fed DoE [130] studied the influence of media composition on solubility for acidic, basic, and neutral drugs. For acidic drugs, the average standardised effect of pH on solubility behaviour was more than three times larger when compared to the other amphiphilic media components. Whilst for basic and neutral drugs the average standardised effect values for pH and amphiphilic components were broadly equivalent with no single component dominant. In addition, a previous SIF study using a four-component mixture design [158] determined that as both pH and TAC increased a general increase in solubility is measured. Therefore, a plot of solubility vs media pH was applied to analyse acidic drugs. Whilst for basic and neutral drugs a plot of solubility versus pH × TAC was used. This latter analysis was also applied to acidic drugs in BCS Class II or IV with higher dose/solubility values.

A representative pH × TAC plot is presented in Figure 25 that highlights how the media recipes were structured in pairs along the axes of an ellipse by the multidimensional analysis. Media 1 and 2 were based on the major axis of the multidimensional ellipse that characterised the FeHIF data cloud [126], while media points 3 and 4 were based on the minor axis. Media points 5 and 6, as well as 7 and 8, were calculated based on additional major and minor axes in other dimensions. The eight media points collectively account for > 95% of the compositional variability observed in the HIF samples for the analysed media components.

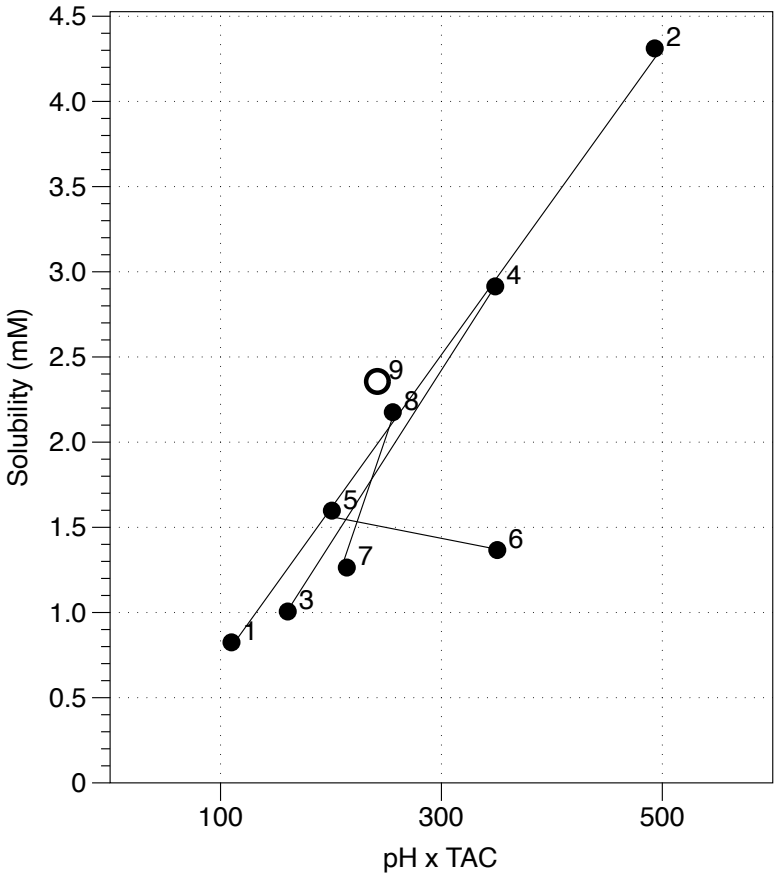


Figure 25. **Representative plot of solubility vs (pH x TAC)**

Note: Point label indicates the media number. Media composition and order as displayed in Table 7.

5.3.2. Acidic Drugs

5.3.2.1. Solubility behaviour

Figure 26 presents solubility plots for the acidic drugs. An easily spotted characteristic is that overall solubility increases with increasing media pH (Figure 26a) and for the majority of drugs media 2 with the highest pH (6.59) presents the highest solubility value. The lowest solubility is measured in media 1 or 7 with the lowest pH value, both at 5.97. The pKa values for most drugs in this study (Table 8) are lower than the lowest media pH (Table 7), confirming that the solubility measured is controlled by the ionised form. This is reinforced by the fitting of a mono-exponential curve through the data and the generally high correlation coefficient for each drug (Figure 2a). The exceptions are phenytoin (see Table 57 and Figure 26b) with a pKa above the highest media pH value and piroxicam with a pKa higher than the lowest media pH but still within the media pH range. Although pH is clearly the driving force for solubility (Figure 26a), there are minor variations in the solubility of

points with close pH values, probably due to the influence of other media components present at high concentrations in the fed state. In some cases the solubility ranking of media 5, 6, and 9 varies despite having similar pH values (6.24, 6.32, and 6.26, respectively). Analogous behaviour is also noted in the ranking of media 2, 8 and 4 (6.59, 6.59, and 6.42, respectively) where for some drugs media 4 with slightly lower pH but highest TAC, is exhibiting higher solubility values than media 8. The lower correlation values calculated for ibuprofen, indomethacin and valsartan might be an interesting indicator of the influence of amphiphilic media content on their solubility, see next paragraph. The behaviour of valsartan is anomalous, since media 5 displays the highest solubility, indicating that amphiphilic component solubilisation is important for this drug, see next section. This pH solubility dependent behaviour is consistent with a published fed state DoE [130], similar to the fasted state [160, 203] and for the purposes of this paper described as Category 1 in Table 15.

Figure 26b presents the solubility plots for the acidic drugs with higher dose/solubility values (mefenamic acid, phenytoin and zafirlukast) in the DCS II or IV range. A comparison of the pH and pH × TAC plots (Figure 26b) for these drugs indicates that amphiphile content and composition might be influencing the solubility behaviour indicated by a lower mono-exponential pH correlation coefficient for these drugs than those presented in Figure 26a. This solubility behaviour is similar to the fasted state [160, 203] and for the purposes of this paper has been described as Category 2 in Table 15.

Table 15. **Biorelevant Fed Simulated Intestinal Fluids - Solubility Behaviours**

Category	**1** **pH controlled TAC variation evident**	**2** **pH & TAC controlled**	**3** **Minimal pH & TAC control**	**4** **pH & TAC & Drug controlled**
Solubility Behaviour	Solubility increases with increasing pH, impact from amphiphilic media components at solubility extremes	Solubility increases with increasing pH and total amphiphile content, solubility behaviour controlled by individual drug interactions with media components	Minimal impact of media components on solubility	No evident solubility relationship between pH and total amphiphile content, drug dependent behaviour, increasing pH and total amphiphile content might reduce solubility

Description	Acidic drugs pKa <8.33[A]	Basic and neutral drugs weak acidic drugs pKa > 8[B]	Neutral drugs[C] Solubility Ratio < 3	Basic and neutral drugs – categorisation based on solubility behaviour
Drugs	Furosemide, ibuprofen, indomethacin, mefenamic acid, naproxen, phenytoin, piroxicam, valsartan, zafirlukast	Aprepitant, carbamazepine, carvedilol, dipyridamole, felodipine, griseofulvin, itraconazole, phenytoin, posaconazole, tadalafil,	Acyclovir, atazanavir, fenofibrate, paracetamol	Atazanavir, bromocriptine, probucol
Comment	Five out of nine examples from non-steroidal anti-inflammatory therapeutic category, expansion into other therapeutic modalities required	Varied physicochemical properties, increased drug examples required	Increased drug examples required	Insufficient data for conclusive analysis, increased drug examples required
Lowest Solubility Media[D] Number and Frequency	1 or 7 (pH = 5.97) 89% 8 out of 9 examples	1 70% 7 out of 10 examples	1 25% 1 out of 4 examples	Not assigned
Highest Solubility Media[D] Number and Frequency	2 (pH = 6.59) 89% 8 out of 9 examples	2 90% 9 out of 10 examples	2 75% 3 out of 4 examples	Not assigned
Mean Solubility Ratio[E] (Highest/Lowest) ± Standard Deviation/Ratio Range	7.28 ± 3.27/7.79 (n = 9)	5.78 ± 1.87/5.45 (n = 10)	1.76 ± 0.92/1.96 (n = 4)	6.43 ± 3.76/7.41 (n = 3)

TAC Total Amphiphile Concentration. A: Based on highest pKa of acidic drugs measured – phenytoin. B: Based on the single example of phenytoin. C: Category could include acidic and basic drugs that have pKa values outside of the media pH ranges. D: Values not equal to Figure 4 or 9, consult drugs list for values included in each category. E: Calculated solubility ratio (highest solubility/lowest solubility).

Figure 2a. Acidic Drugs I

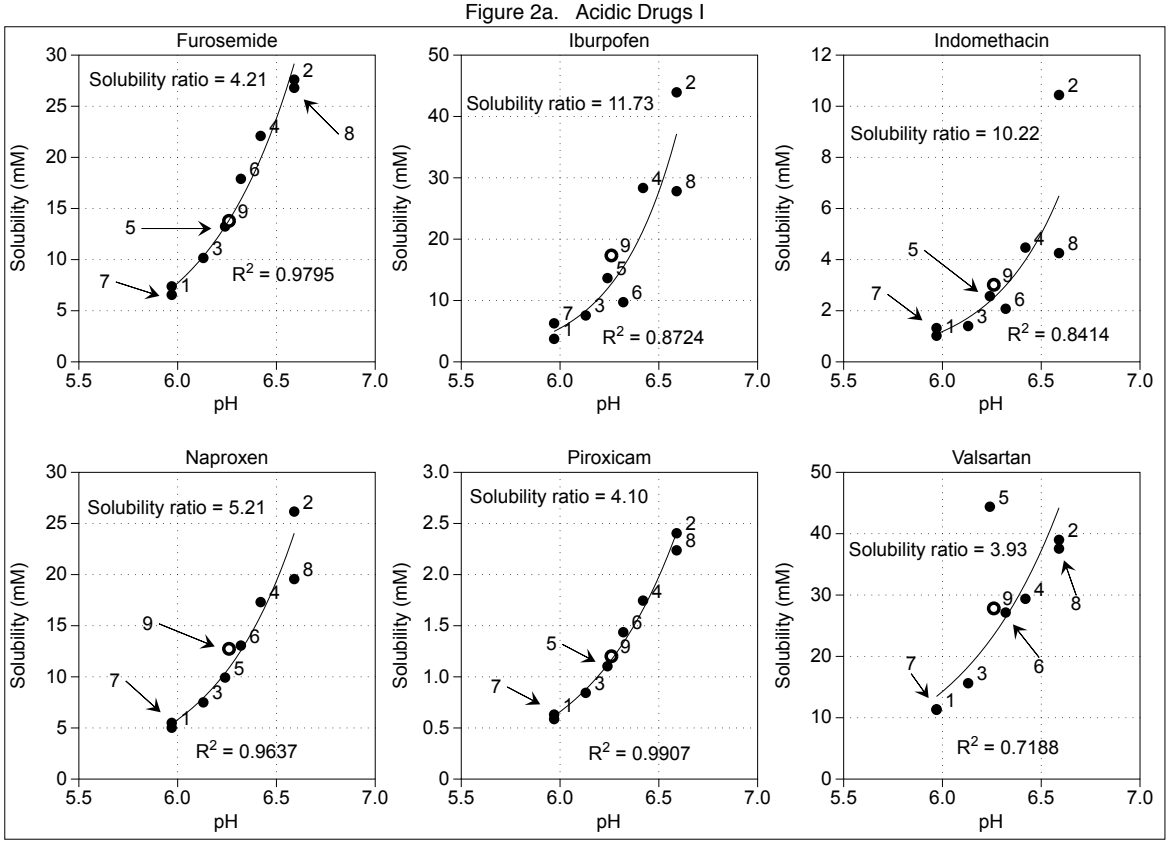


Figure 2b. Acidic Drugs II

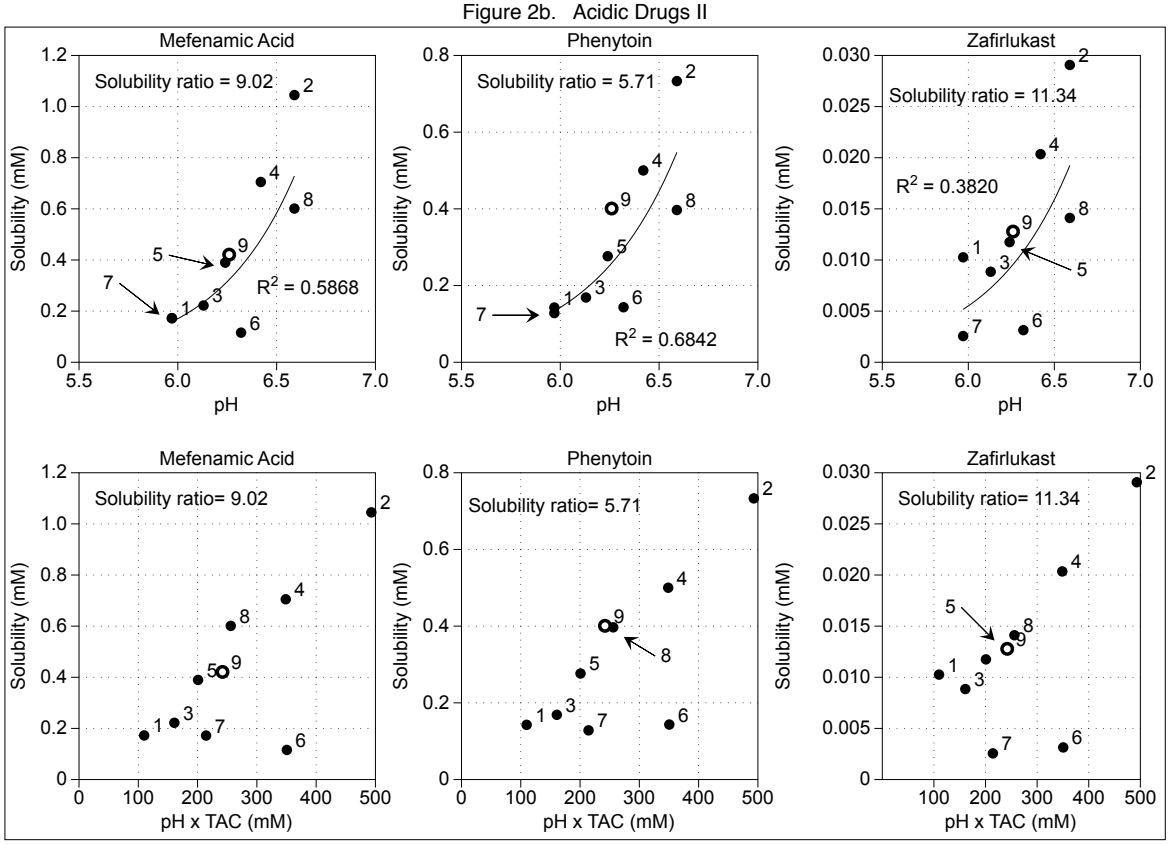


Figure 26. **(a) Acidic Drug Solubility vs Media pH; (b) Acidic Drug Solubility vs Media pH and Media pH x TAC**

Note (a): Point label indicates media number. Media composition and order as displayed in Table 7. Line mono-exponential best fit of solubility vs pH, solubility = Aek x pH where A = constant, k = power value and pH = media pH

value. R2 = correlation coefficient of fitted exponential. Note (b): Point label indicates media number, see Table 57. pH figures (top graphs) Line mono-exponential best fit of solubility vs pH, solubility = Aek x pH where A = constant, k = power value and pH = media pH value. R2 = correlation coefficient of fitted exponential.

5.3.2.2. Solubility behaviour analysis

The solubility behaviour in this study is in line with previous literature [204] and in fed DoE studies [123, 130, 132] reporting acidic drug solubility in FeSSIF. The most significant factor found to affect solubility was pH with a clear trend that solubility increased with media pH. Similar behaviour was registered for the fasted state [203] which is consistent with the fasted DoE study [160] that determined the impact of pH on solubility to be twenty times greater than any media amphiphilic component. The fed state DoE [130] found the impact of pH on acidic drugs to be dominant but less predominant than in the fasted state. Media pH still plays a major role in fed state, but oleate, bile salts and their interactions also play an important secondary role for certain acidic drugs [130]. This variation between fasted and fed states is probably related to the higher concentrations of amphiphilic components present in the fed state and the impact of these components might explain the variations in media ranking that were observed for some acidic drugs in this paper. In this study phenytoin and zafirlukast (Figure 26b) were more affected by media amphiphilic components than other acid drugs with media 2 presenting higher solubility values than expected by pH alone. For these drugs the fed DoE [130] found that their solubility was positively affected by pH, oleate and lecithin which is consistent with this study's observations. Ibuprofen, indomethacin and valsartan also present a slight variation in the media ranking which is in line with the DoE analysis since their solubility was also found to be affected by pH and bile salts. Mefenamic acid and furosemide were not studied in the DoE study and their solubility cannot be analysed in a similar fashion. Overall, the dominant impact of pH on acidic drug solubility is evident and the exceptions regarding the impact of amphiphilic content on the drugs studied are consistent with the fed DoE [130]. Of note is that the average solubility ratio in this fed state study (Category 1 drugs Table 15: 7.28 ± 3.27, mean ± standard deviation, n = 9) is lower than the comparable fasted study (23.4 ± 11.8, n = 7) [203], reflecting the narrower media pH range in the fed (pH 5.97 – 6.59, Δ = 0.62) compared with the fasted state (pH 5.72 – 7.34, Δ = 1.62). If this effect is present in vivo then the pharmacokinetic variability for acidic drugs in the fasted state might be larger than in the fed state.

5.3.2.3. Media frequency analysis

Figure 27 presents the frequency of each media recipe as the highest and lowest solubility value for acidic drugs. The highest solubility was provided by the media with highest pH, media 2 (Table 57), in 8 out of 9 drugs (89%). The only exception was valsartan where the highest solubility was measured in media 5. The pH difference between the media is not large (Table 5 7) and the solubility difference, (media 5 = 44 ± 0.74 mM and media 2 = 39 ± 1.6mM: all values mean ± standard deviation n = 3) if compared using a non-parametric Mann-Whitney test is not significant (P = 0.10), see below.

The lowest solubility in 5 out of 9 drugs (56%) was measured in media 1. For 3 (33%) drugs phenytoin (media 1 = 0.14 ± 0.0072mM and media 7 = 0.13 ± 0.018 mM), piroxicam (media 1 = 0.63 ± 0.015 mM and media 7 = 0.59 ± 0.0095 mM) and zafirlukast (media 1 = 0.0010 ± 0.00051 mM and media 7 = 0.0026 ± 0.00039 mM) it was media 7. In one case mefenamic acid the lowest solubility is measured in media 6 (media 1 = 0.17 ± 0.0062 mM and media 6 = 0.12 ± 0.017 mM). As above a non-parametric Mann-Whitney comparison of the solubility data sets for the four drugs is not significant (P = 0.10), see below. The lowest solubility media is predominated by the lowest pH media (media 1 56%) but other media (media 7 and 6) contribute 4 out of 9 (44%) results. These media have very similar pH values (1 = 5.97; 3 = 6.13; 6 = 6.32; 7 = 5.97) and the major difference between them is the total concentration of amphiphiles present (1 = 18.4 mM; 3 = 26.2 mM; 6 = 55.5 mM; 7 = 35.9 mM). This can be visualised in Figure 26b with the pH × TAC plots where media 1, 3, 6 and 7 all have a low solubility. A previous study [158] noted that for indomethacin a high amphiphile concentration depressed solubility, albeit at a higher media pH of 7. The result noted in this study is similar and may indicate that high amphiphile concentrations can depress solubility for acidic drugs a behaviour that was not evident in the DoE study.

The statistical comparison of the data above does not detect any significant difference in the measured solubility values. This indicates that the use of media 1 to determine the lowest solubility is appropriate however, this result requires a cautious interpretation. In previous studies with larger data sets [123, 131] SIF measured solubility values were not normally distributed and therefore a non-parametric statistical comparison was valid. Application of non-parametric analysis in this study to compare two individual media might not be appropriate, however with only 3 measurements calculation of the solubility distributions statistical properties is not feasible. Further examination of this issue is required to fully assess individual media solubility behaviour and comparison.

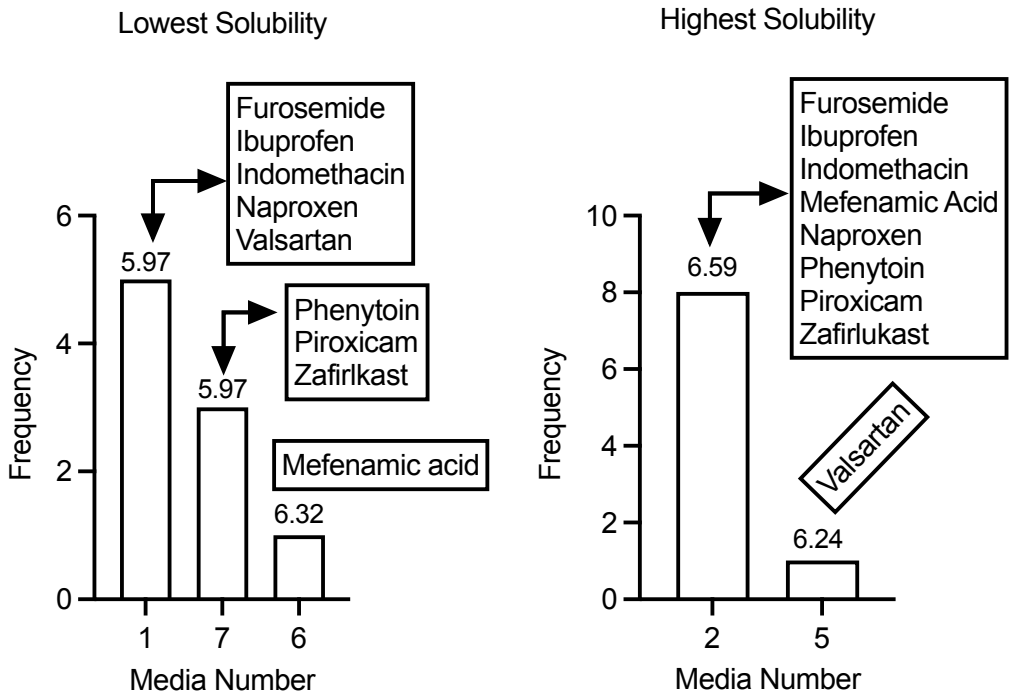


Figure 27. **Frequency of lowest and highest solubility media for acidic drugs**

Note: Drugs as listed in boxes, number on bar = media pH.

5.3.3. Basic and Neutral Drugs

5.3.3.1. Solubility behaviour

Figure 28a and Figure 28b and Figure 29a and Figure 29b, present the solubility plots for the basic and neutral drugs respectively. Figure 30 and Figure 31 re-present the data as a spider or polar plot where solubility values have been normalised to the highest value (set to 100) and arranged in a clockwise order starting at 12 o'clock with the lowest pH × TAC media value (Table 57, media 1) and continuing to the highest value (media 2).

Based on a visual analysis of Figure 26a and Figure 28a, it can be observed that for these drugs (basic - aprepitant, carvedilol, dipyridamole, posaconazole, tadalafil; neutral - carbamazepine, felodipine, fenofibrate, griseofulvin, itraconazole, and paracetamol) there is a solubility pattern with media 1 generally providing the lowest solubility values and media 2 the highest. In some cases (basic - carvedilol, posaconazole; neutral - paracetamol) media 5, 6, or 7 provide the lowest solubility. The intermediate media have an increasing solubility and similar but not a consistent pattern across all drugs. Minor variations in the intermediate media ranking can be observed for the majority of drugs highlighting the influence of media composition on solubility [130, 157]. In Figure

26b and Figure 28b the drugs do not exhibit this pattern and a complicated solubility behaviour is evident.

Analysis of the spider plots highlights that the majority of basic (aprepitant, carvedilol, dipyridamole, posaconazole and tadalafil) and neutral (carbamazepine, felodipine, fenofibrate, griseofulvin and itraconazole) drugs display a similar shape profile and increasing solubility clockwise around the web from media 1 to media 2. The increase is not smooth and there is variation in the intermediate media as discussed above, but the shape highlights their pH × TAC solubility dependency. Atazanavir and bromocriptine (Figure 30) exhibit different behaviours with a distinctive waisted plot shape and for neutral drugs, exceptional behaviour is registered for probucol, acyclovir and paracetamol with the latter two displaying almost circular spider plots.

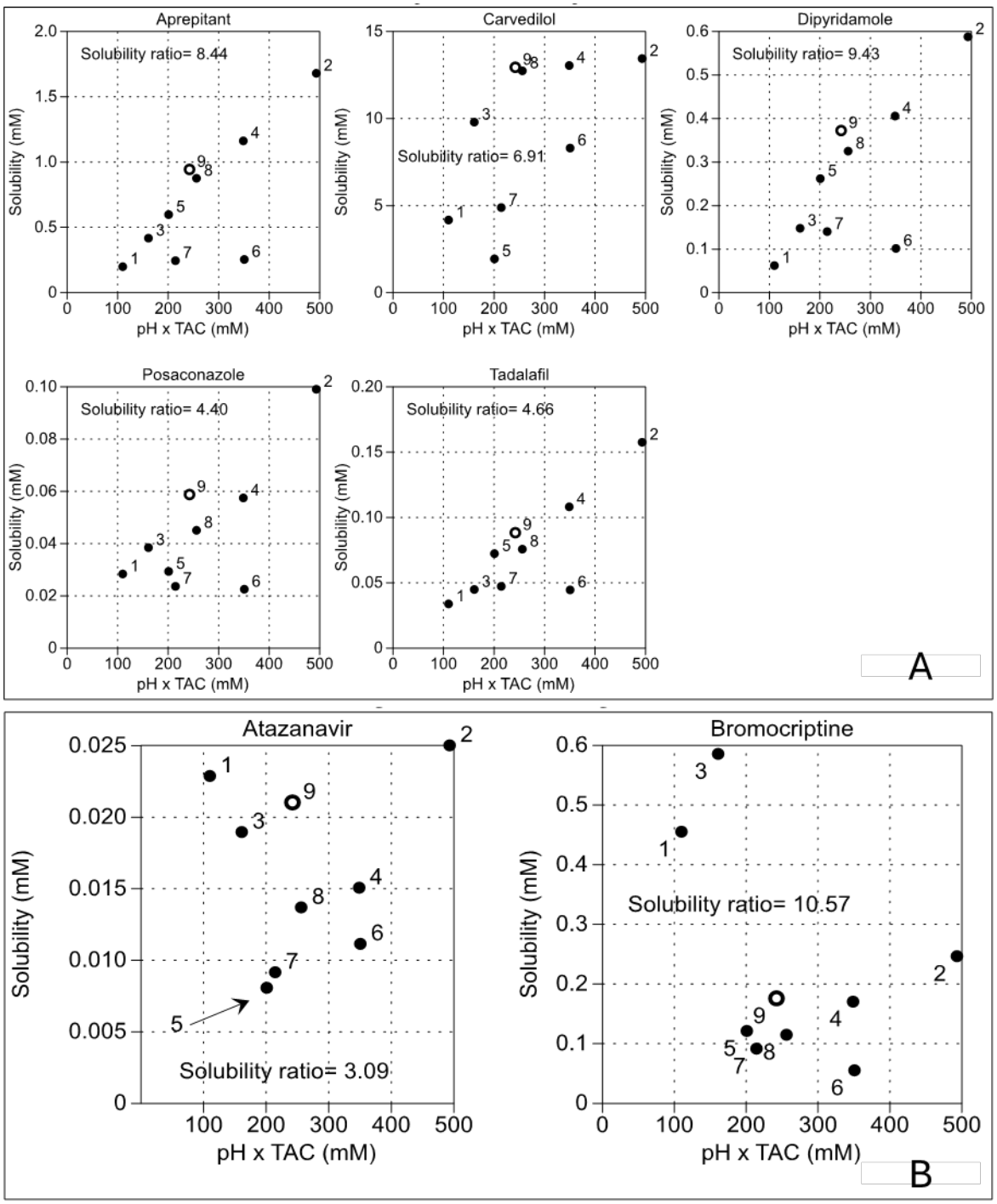


Figure 28. **(a) Basic Drug Solubility vs Media pH x TAC. (b) Basic Drug Solubility vs Media pH x TAC**

Note: Point label indicates media number. Media composition and order as displayed in Table 7.

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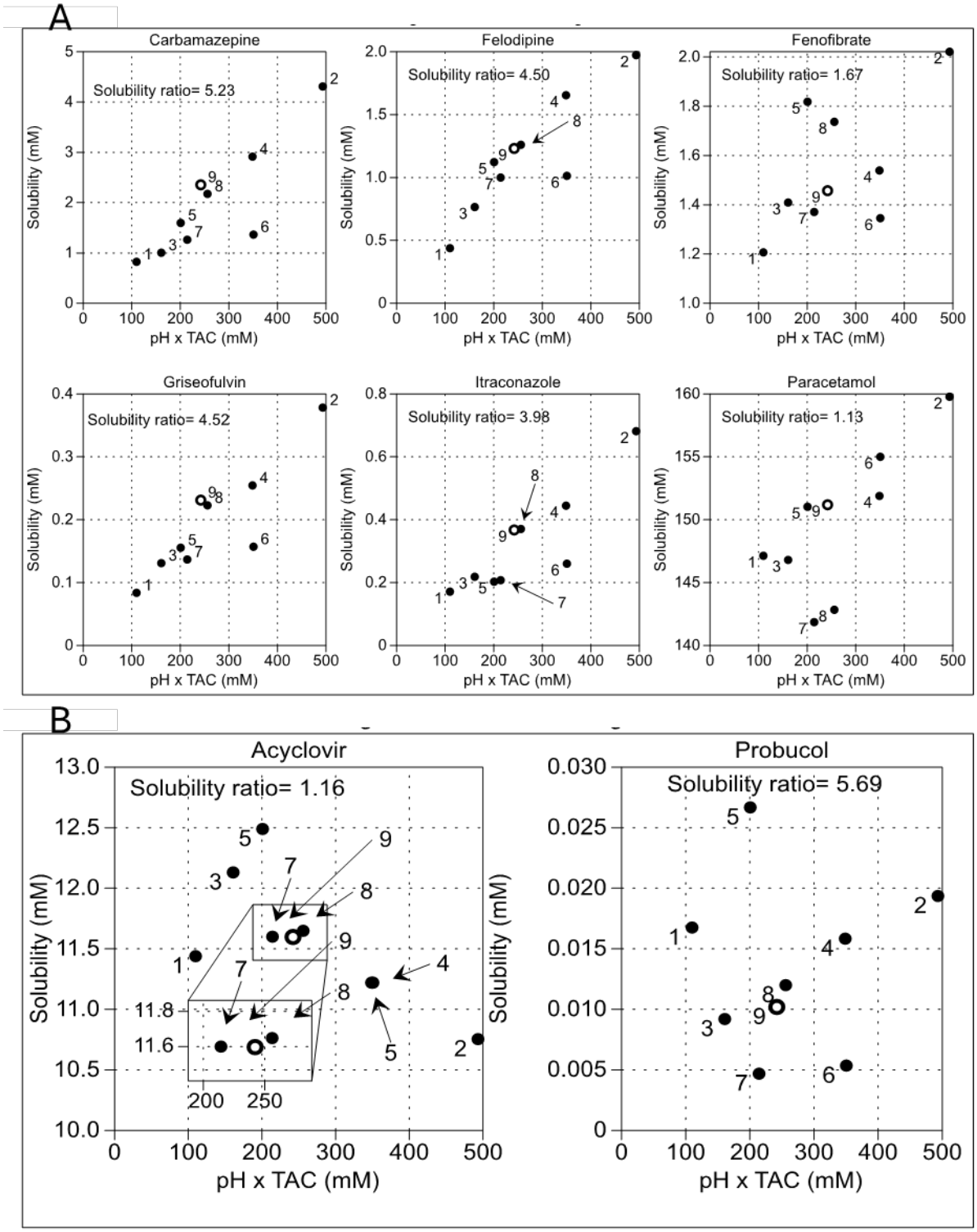


Figure 29. **(a) Neutral Drug Solubility vs Media pH x TAC, (b) Neutral Drug Solubility vs Media pH x TAC**

Note: Point label indicates media number. Media composition and order as displayed in Table 7.

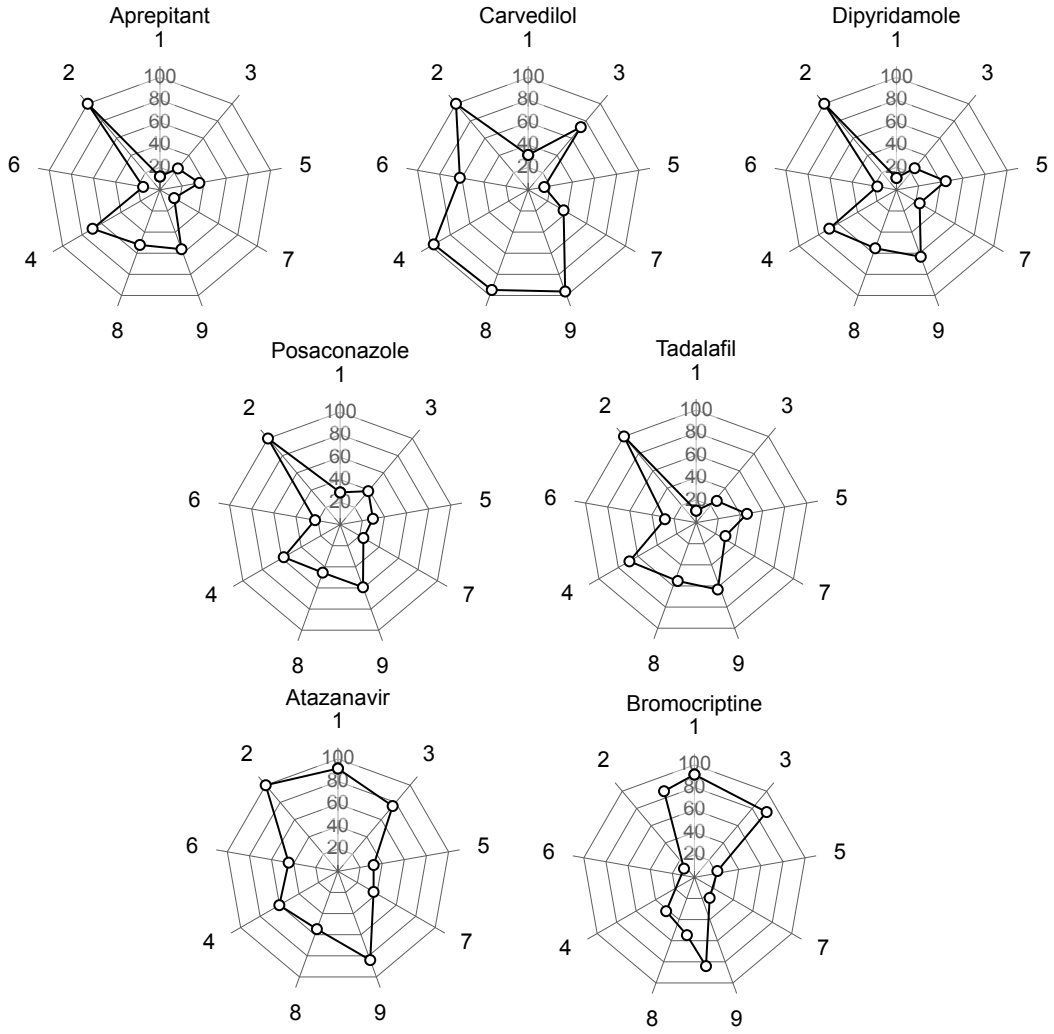


Figure 30. **Basic Drugs – Solubility Spider Plot**

Note: Highest solubility value normalised to 100; points correspond to media number (Table 7) arranged in a clockwise order of increasing pH × TAC – lowest value at 12 o'clock.

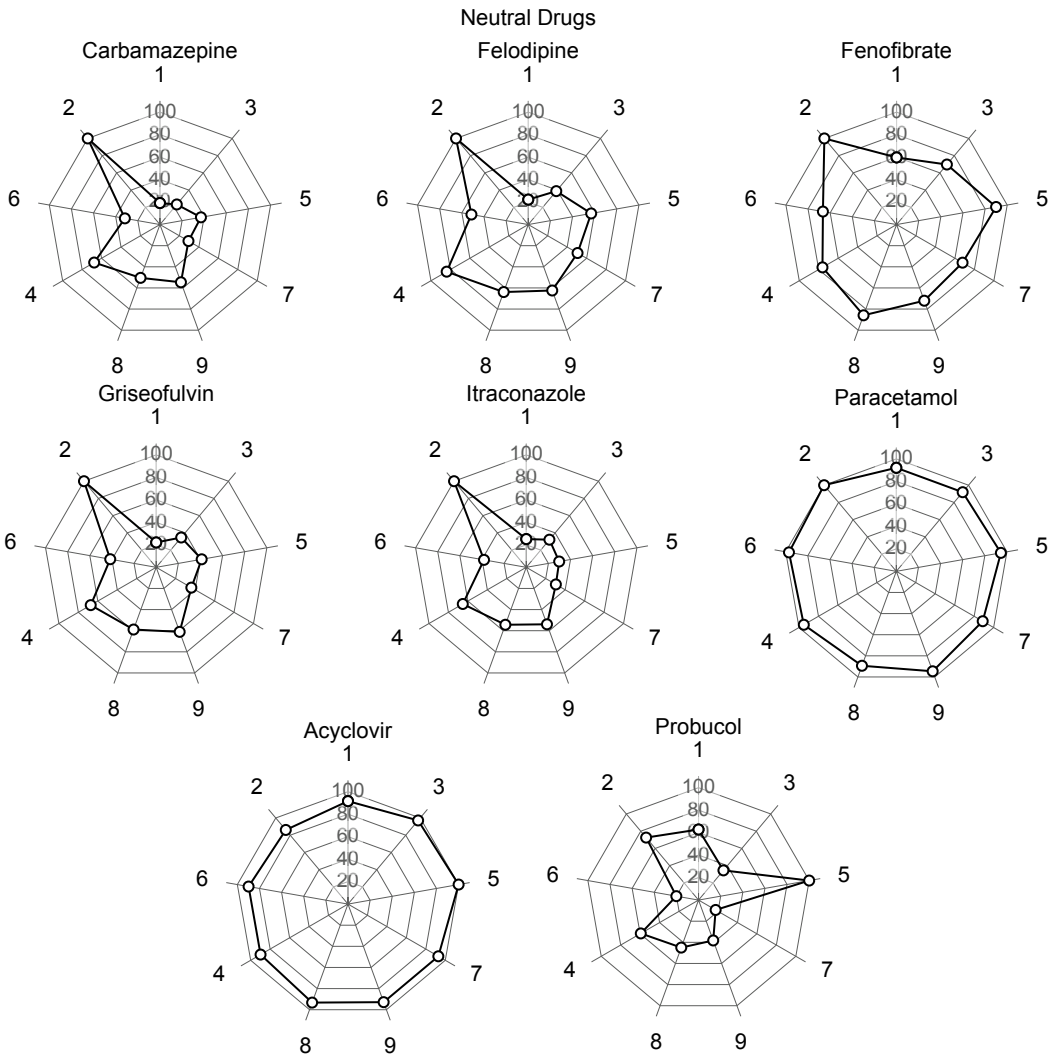


Figure 31. **Neutral Drugs – Solubility Spider Plot**

Note: Highest solubility value normalised to 100; points correspond to media number (Table 7) arranged in a clockwise order of increasing pH × TAC – lowest value at 12 o'clock.

5.3.3.2. Solubility behaviour analysis

The solubility behaviour registered for the basic and neutral drugs in this experiment is in broad agreement with the behaviour in previous fed DoE studies [123, 130-132]. When examining the solubility versus pH × TAC distributions for basic drugs it is clear the impact of pH is not as prominent as with acidic drugs. The previous DoE study [130] found a more intricate solubility relationship is apparent for basic drugs where on average pH, oleate, and bile salts display comparable effects on solubility, with lecithin having a lower effect. This finding is consistent with the behaviour displayed by the majority of basic drugs in this study. Neutral drug solubility behaviour was found on average to be influenced primarily by oleate and bile salts and to a lesser extent by lecithin and pH [130]. Since pH cannot influence neutral drug ionisation, the effect is

conveyed through the ionisation of media components. This mechanism is similar to the one observed in the fasted DoE [160].

For both basic and neutral drugs, there is an overarching trend towards increased solubility with increasing media pH × TAC, which is why media 1 generally has lower solubility compared to media 2 (Figure 26a and Figure 28a). However, for each drug this trend is subject to modification by the standardised effect of each media component on individual drug solubility [130]. For aprepitant, carvedilol and tadalafil, oleate and bile salt were media components significantly positively influencing solubility, pH was only significant for aprepitant and carvedilol whilst lecithin was minimally significant for these drugs. This explains the similar polar plot shapes for these drugs. For bromocriptine no media components significantly influenced solubility, which explains the different solubility profiles in Figure 26b and Figure 30. Dipyridamole and atazanavir were not studied in the fed DoE therefore no comparative analysis regarding the significant effect of media components on solubility is available. However, dipyridamole seems to behave in a similar manner to the majority of basic drugs with its solubility appearing to be linked to pH × TAC. Atazanavir presents an unusual behaviour with media 1 (lowest pH × TAC) exhibiting a similar solubility value to media 2 (highest pH × TAC) revealing no correlation between solubility and pH × TAC. Atazanavir's polar plot shape is similar to bromocriptine and this implies that for atazanavir none of the media components have a significant influence on solubility. For felodipine, fenofibrate, itraconazole and probucol, oleate, bile salt and lecithin were media components significantly positively influencing solubility, but to very different magnitudes. The exception was fenofibrate where bile salt had a negative impact on solubility [130]. The impact of pH was variable, being negative for itraconazole and probucol solubility, not significant for fenofibrate and positive for felodipine. Felodipine, fenofibrate and itraconazole have very similar solubility profiles, whilst probucol has a different distinctive profile. Probucol's behaviour can be rationalised based on the very high solubilisation effect of bile salt in the DoE, and media bile salt concentration in combination with other components. Acyclovir, carbamazepine, griseofulvin and paracetamol were not studied in the fed DoE and no analysis of media components on solubility is available. Carbamazepine and griseofulvin display congruent solubility behaviour to felodipine, fenofibrate and itraconazole and therefore can be assumed to show similar interaction with media components.

For acyclovir and paracetamol (also fenofibrate) the spider plots (Figure 31), display solubility that does not vary with media composition and therefore measured solubility ratios are low at 1.16, 1.13, and 1.67 respectively. A previous fasted study [203] also registered similar solubility behaviour for acyclovir and paracetamol (solubility ratios 1.15 and 1.22) thus confirming that solubility is not

influenced by the different composition of these fasted and fed media. Fenofibrate's solubility ratio has decreased from the fasted to fed state (7.65 vs 1.67), whilst for griseofulvin the reverse is evident (2.32 vs 4.52). This is an interesting biopharmaceutical observation that may be responsible for differences in pharmacokinetic behaviour between the fasted and fed states and worthy of further investigation.

Overall, for basic and neutral drugs three solubility behaviours are identifiable: solubility dependent on the variation of pH × TAC (Category 2, Table 15), limited solubility impact of media variation where solubility ratio is ≤ 3 (Category 3, Table 15) and no correlation between pH × TAC variation and solubility (Category 4, Table 15).

5.3.3.3. Media frequency analysis

Figure 32 presents the frequency of each media recipe as the lowest and highest solubility value for basic and neutral drugs. For basic drugs the highest solubility was measured in media 2 (Table 57) in 6 out of 7 drugs (86%). The only exception was bromocriptine, discussed previously, where the highest solubility was provided by media 3. The lowest solubility was measured in media 1 (Table 57) for 3 drugs (aprepitant, dypridamole and tadalafil) (43%), in media 5 for 2 (atazanavir and carvedilol) and media 6 for 2 (bromocriptine and posaconazole) out of 7 (29%). For carvedilol and posaconazole the solubility difference between media 1 and the measured lowest media is small (carvedilol 1 = 4.17 mM ± 0.236; 5 = 1.95 mM ± 0.0641: posaconazole media 1 = 0.0284 mM ± 0.00257; 6 = 0.0225 mM ± 0.00229), As above a non-parametric Mann-Whitney comparison of the solubility data sets for the four drugs is not significant (P = 0.10). Therefore for these drugs media 1 would represent an approximate value for the lowest fed state solubility. Atazanavir and bromocriptine were discussed above (Section 5.3.3.1) as they present a very different solubility behaviour from the other basic drugs. For these drugs there is no obvious signal to this behaviour other than the polar plot shape and for these drugs identifying the lowest solubility media in the fed state may require measurement of all media. For neutral drugs the lowest solubility was registered in media 1 for 5 out of 8 drugs (63%), in media 7 for probucol and paracetamol and in media 2 for acyclovir. Paracetamol and acyclovir have very low solubility ranges (see above), with almost circular polar plots with minimal media impact on solubility. Therefore media 1 would represent the lowest solubility for 7 out of 8 drugs (88%) with minimal error. A similar argument will apply for these drugs to the identification of the highest solubility media, which was identified as

media 2 for 7 out of 8 drugs (88%). The behaviour of probucol as discussed above is individualistic and only identifiable via the polar plot shape.

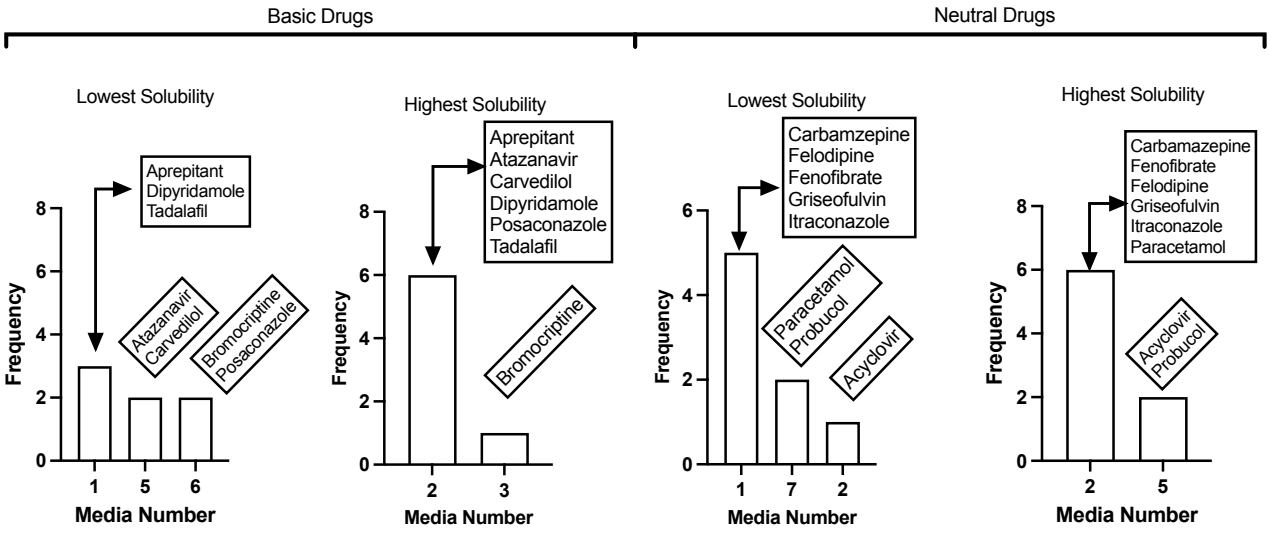


Figure 32. **Frequency of lowest and highest solubility media for basic and neutral drugs.**

Note: Drugs as listed in boxes.

5.4. Conclusions

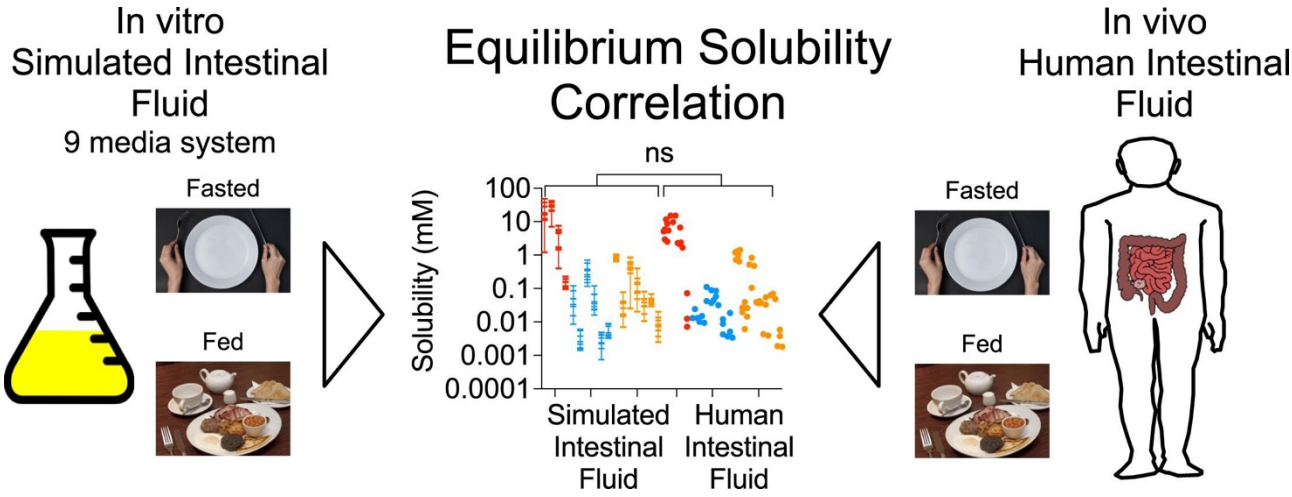
In this study 24 drugs were examined to assess solubility behaviour in 9 fed state simulated intestinal media with a biorelevant composition determined by a multidimensional analysis of sampled fed human intestinal fluid. The caveat mentioned in the introduction regarding the use of a liquid meal, Ensure Plus™ which is not equivalent to solid meals, to attain the fed state in the original study [159] utilised to derive the media in this study is worthy of repetition. The solubility behaviour for the three categories of drugs acidic, basic and neutral is consistent with previous DoE studies examining simulated fed state intestinal media [123, 130, 131]. For acidic or category 1 drugs (furosemide, ibuprofen, indomethacin, mefenamic acid, naproxen, phenytoin, piroxicam, valsartan and zafirlukast) solubility is pH-dependent. For the majority of basic, neutral and weakly acidic drugs (aprepitant, carbamazepine, carvedilol, dipyridamole, felodipine, griseofulvin, itraconazole, phenytoin, posaconazole and tadalafil) solubility is controlled by media pH × TAC (category 2), with generally increasing solubility as pH × TAC increases. Solubility variation is evident due to the diversity of individual drug interactions with media components [130, 157, 158]. For some drugs (acyclovir, atazanavir, fenofibrate, paracetamol) there is a very low solubility variation (category 3)

across all the measured media. Three drugs (atazanavir, bromocriptine, probucol, category 4) exhibit an unusual solubility behaviour that does not conform with previous categories.

Overall a structured solubility behaviour has been identified for 18 of the 24 drugs studied with media 1 identifying the lowest solubility in 80% of cases and media 2 the highest solubility in almost 90% of cases. For 4 of the remaining drugs (acyclovir, atazanavir, fenofibrate, paracetamol) their minimal solubility variation means that media 1 and media 2 would still provide a realistic solubility assessment. 3 drugs (atazanavir, bromocriptine and probucol) present individualistic solubility behaviour that is at this stage not simply characterised. This study demonstrates for the majority of drugs the fed solubility range can be identified *in vitro* through application of only 2 media. In combination with the previous fasted study [203] this provides very interesting possibilities during drug discovery and development to determine fasted and fed solubility envelopes and indicates that the multidimensional media system [126] is worthy of further investigation.

Chapter 6

A novel simulated media system for *in vitro* evaluation of bioequivalent intestinal drug solubility



This chapter includes the full text of the published article from the European Journal of Pharmaceutics and Biopharmaceutics. (Abuhassan, Q., Silva, M. I., Tamimi, R. A.-R., Khadra, I., Batchelor, H. K., Pyper, K., & Halbert, G. W. (2024). A novel simulated media system for *in vitro* evaluation of bioequivalent intestinal drug solubility. European Journal of Pharmaceutics and Biopharmaceutics, 199, 114302. https://doi.org/https://doi.org/10.1016/j.ejpb.2024.114302) including its introduction, methods, results and conclusions.

For this work, I carried out all experiments for the fed state, performed the data analysis and graph plotting and contributed with the fed part of the manuscript draft.

6.1. Introduction

Oral drug administration is preferred by patients but solid drug must dissolve in the gastrointestinal tract (GIT) to enable absorption and produce a response. Intestinal solubility controls [138] absorption and the DCS [174] links intestinal solubility, volume and dose administered with permeability to classify absorption behaviour. Most drug development candidates are poorly soluble (DCS Class II and IV)[205] and during drug discovery and development an accurate *in vitro* intestinal solubility measurement is essential to assess in vivo biopharmaceutical properties[206] and potential formulation strategies.

The gold standard for measuring intestinal solubility is sampled HIF [174]. However, HIF is a multicomponent system containing in the fasted (Fa) state endogenous solubilising agents e.g. bile salts and phospholipids, with in the fed (Fe) state additional food digestion products such as fatty acids and glycerides [159]. Average bile salt concentration varies from 3mM in the fasted state to 15mM in the fed increasing drug solubility and absorption, leading to a potential "food effect" [207]. This prandial variation is superimposed on intra and inter subject variability [126, 159], along with population and disease changes[208]. Obtaining HIF requires nasogastric intubation, only provides small volumes (1-2mL) and exhibits intra and inter subject variability [159]. Drug solubilities measured in sampled Fa/FeHIF are therefore due to HIF compositional variability highly variable[150] and single values are difficult to correlate to in vivo biopharmaceutical performance.

To mitigate HIF availability, fasted and fed simulated intestinal fluid (Fa/FeSIF) based on average HIF component values was introduced as an *in vitro* surrogate. Several versions were developed [155] by comparing drug Fa/FeHIF solubilities vs Fa/FeSIF and adjusting SIF media composition. However, there is solubility variability between Fa/FeSIF recipes [176] and between Fa/FeHIF samples and therefore no consensus on the optimal Fa/FeSIF media.

Statistically guided studies on SIF composition and solubility [130, 160] identified the media components driving solubility either individually or in combination [158]. These studies also revealed that drug molecular structure and physicochemical properties influence solubility variability in combination with media variability [158]. Due to these inherent properties of the drug and the media intestinal solubility is therefore a range. A single solubility value determined in a sampled (Fa/FeHIF) or fixed simulated intestinal media (Fa/FeSIF) composition is therefore incapable of representing the potential in vivo solubility range (which can vary by orders of magnitude) due to HIF variability [208].

To capture HIF compositional variability and therefore solubility variability, a study [126] reported a five dimensional (pH, bile salt, phospholipid, free fatty acid and cholesterol) analysis of Fa/FeHIF samples [159]. The dimensions or media constitutents included were those that had the major individual impact on drug solubility[130, 160]. For both prandial states, eight intestinal media that incorporated 95% of HIF compositional variability were determined along with a centre point (Fa9SIF and Fe9SIF). Each media is a novel FaSIF[127] or FeSIF[200] directly linked to Fa/FeHIF composition with all 9 in combination covering 95% of either the fasted or fed compositional variability. There is a fed state limitation since the original study[159] administered the liquid feed Ensure Plus™, which is not equivalent to solid meals.

Previous studies have compared Fa/Fe9SIF solubility[127, 200] to DoE studies[123, 130, 132, 160], the DCS[174] with calculation of a new solubility population distribution[161, 201] and to determine structured solubility behaviour[203, 209] that identifies the lowest and highest solubility media. Due to Fa/Fe9SIF's derivation[126] from Fa/FeHIF composition [159], measured drug solubility ranges should be bioequivalent and include measured Fa/FeHIF values. In this paper we have compared Fa/Fe9SIF solubility data for twenty three drugs in the fasted and twenty in the fed state to published Fa/FeHIF solubilities (see Table 16 and Table 17). Establishing an *in vitro in/ex vivo* intestinal solubility correlation along with the ability to determine a drug's intestinal solubility variability will introduce a transformational change throughout drug discovery, development and formulation [206].

Table 16. **Sampled Fasted HIF Literature Equilibrium Solubility Values**

Drug	Solubility (mM)	Time (hours)	Temp (°C)	Solid/ Volume	Separation	HIF Sample Location	HIF Sample Number	Age	Pooled	Reference
Acidic Drugs										
Furosemide	5.862	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	11.62	30	23	NA/0.25ml	Centrifugation	Duodenum	5	24-39	Y	[210] from [150]
	8.76	24	37	NA/0.2ml	Centrifugation	Jejunum	10	NA	Y	[181]
	5.44	24	37	NA/0.2ml	Centrifugation	Ileum	10	NA	N	[181]
	2.95	3	37	2mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	2.52	3	37	2mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	5.49	3	37	2mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	5.13	3	37	2mg/ml	Centrifugation	Jejunum	5	22-35	N	[187]
Ibuprofen	9.65	30	23	NA/0.25ml	Centrifugation	Duodenum	5	24-49	Y	[210] from [150]
	15.1	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	15.1	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Indomethacin	1.677	NA	NA	NA	NA	NA	NA	NA	NA	[211]
	2.368	NA	37	NA/0.5ml	Centrifugation	Duodenum	5	21-37	N	[204]
	2.151	24	37	0.5mg/0.5ml	Centrifugation	Duodenum	8	18-25	N	[212]
	2.301	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	6.658	30	23	NA/0.25ml	Centrifugation	Duodenum	5	24-39	Y	[210] from [150]
Naproxen	7.148	30	23	NA/0.25ml	Centrifugation	Duodenum	5	24-39	Y	[210] from [150]
Phenytoin	0.00721	3	37	2mg/ml	Centrifugation	Jejunum	5	22-35	N	[187]
	0.0719	3	37	2mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	0.0125	3	37	2mg/ml	Centrifugation	Jejunum	5	22-35	N	[187]
Piroxicam	1.198	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	2.454	30	23	NA/0.5ml	Centrifugation	Duodenum	5	24-39	Y	[210] from [150]
Valsartan	11.00	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	10.77	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Zafirlukast	$6.43x10^{-4}$	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	$6.43x10^{-4}$	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Basic Drugs										
Aprepitant	0.0243	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	0.0131	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.0131	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Atazanavir	0.0151	27	37	0.8mg/0.3ml	Centrifugation	Duodenum	4	24-27	Y	[214]
	0.0101	48	37	NA/1ml	Centrifugation	Duodenum	20	NA	Y	[215]
	0.00936[c]	48	37	2mg/1ml	Centrifugation	Duodenum	20	18-31	Y	[216]
Carvedilol	0.0886	24	37	1mg/ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	0.111	30	37	1mg/0.5ml	Centrifugation	Duodenum	11	NA	Y	[217]
	0.037	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.042	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Dipyridamole	0.0446	3	37	45mg/4.5ml	Centrifugation	Duodenum	12	NA	Y	[218]
	0.0575	24	37	1mg/ml	Centrifugation	Jejunum	NA	NA	Y	[213]

Drug	Solubility (mM)	Time (hours)	Temp (°C)	Solid/ Volume	Separation	HIF Sample Location	HIF Sample Number	Age	Pooled	Reference
	0.0317	24	37	NA/0.2ml	Centrifugation	Jejunum	10	NA	Y	[181]
	0.0851	24	37	NA/0.2ml	Centrifugation	Ileum	10	NA	N	[181]
Itraconazole	0.0088	24	37	2mg/0.5ml	Centrifugation	Duodenum	5	20-30	Y	[219]
	0.00425	3	37	2mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	0.012	3	37	2mg/ml	Centrifugation	Jejunum	5	22-35	N	[187]
Posaconazole	0.0051	30	23	NA/0.5ml	Centrifugation	Duodenum	5	20-32	Y	from [150]
	0.00368	48	37	NA/1ml	Centrifugation	Duodenum	20	18-31	Y	[215]
	0.00342[c]	48	37	2mg/1ml	Centrifugation	Duodenum	20	18-31	Y	[216]
	0.0186[a]	48	37	2mg/1ml	Centrifugation	Duodenum	20	18-31	Y	[216]
Tadalafil	0.018	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.020	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
Neutral Drugs										
Carbamazepine	1.422	24	37	0.5mg/0.5ml	Centrifugation	Duodenum	8	18-25	N	[212]
	1.2	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	1.294	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	0.720	30	23	NA/0.25ml	Centrifugation	Duodenum	5	24-39	Y	[210] from [150]
	0.644	3	37	2mg/1ml	Filtration	Duodenum	5	22-35	N	[187]
	1.01	3	37	2mg/1ml	Filtration	Jejunum	5	22-35	N	[187]
	0.767	3	37	2mg/1ml	Filtration	Duodenum	5	22-35	N	[187]
Danazol	0.0197	24	37	0.5mg/0.5ml	Centrifugation	Duodenum	8	18-25	N	[212]
	0.0261	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	0.0391	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	0.0061	>17	37	3mg/5ml	Centrifugation	Jejunum	13	NA	NA	[220]
	0.0267	24	37	1.2mg/1.2ml	Centrifugation	Jejunum	12	24-40	Y	[221]
	0.0145	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
Diazepam	0.473	24	37	0.5mg/0.5ml	Centrifugation	Duodenum	8	18-25	N	[212]
	0.520	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[204]
	0.8344	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[204]
	0.1054	NA	NA	NA	NA	NA	NA	NA	NA	[211]
Felodipine	0.0364	24	37	1.2mg/1.2ml	Centrifugation	Jejunum	12	24-40	Y	[221]
	0.0364	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	0.00343	3	37	1mg/ml	Centrifugation	Duodenum	5	22-35	N	[187]
	0.0416	24	37	2mg/ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.039	24	37	2mg/ml	Filtration	Duodenum	16	18-45	Y	[142]
Fenofibrate	0.0546	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]
	0.0331	24	37	2mg/0.5ml	Centrifugation	Duodenum	4	19-35	Y	[222]
	0.0043	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.0039	24	37	2mg/ml	Filtration	Duodenum	16	18-45	Y	[142]
Griseofulvin	0.0623	24	37	1.2mg/1.2ml	Centrifugation	Jejunum	12	24-40	Y	[221]
	0.0697	24	37	0.5mg/0.5ml	Centrifugation	Duodenum	8	18-25	N	[212]
	0.0482	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
Prednisolone	1.636	5	37	NA/0.2ml	Centrifugation	Jejunal	15	NA	Y	[223]
	1.337	24	37	1mg/0.3ml	Centrifugation	Duodenum	6	20-29	N	[147]

Drug	Solubility (mM)	Time (hours)	Temp (°C)	Solid/ Volume	Separation	HIF Sample Location	HIF Sample Number	Age	Pooled	Reference
Probucol	0.0019	24	37	1.2mg/1.2ml	Centrifugation	Jejunum	12	24-40	Y	[221]
	0.0018	24	37	1mg/1ml	Centrifugation	Jejunum	NA	NA	Y	[213]
	0.0058	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]
	0.0038	24	37	2mg/1ml	Filtration	Duodenum	16	18-45	Y	[142]

A amorphous solid form; c crystalline solid form; NA Information not available.

Table 17. **Sampled Fed HIF Literature Equilibrium Solubility Values**

Drug	Solubility (mM)	Time (hours)	Temp (°C)	Solid/ Volume	Separation	HIF Sample Location	Collection Time (min)	Meal Type	HIF Sample Number	Age	Pooled	Ref
Acidic Drugs												
Furosemide	6.386	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
	5.497	24	37	NA/0.5ml	Centrifugation	Jejunum	15-120	Ensure +	8	18-25	Y	[212] from
	5.442	24	37	NA/0.2ml	Centrifugation	Ileum	NA	NA	10	NA	N	[175]
												[181]
Ibuprofen	12.28	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	11.90	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Indomethacin	1.954	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
	1.817	24	37	NA/0.5ml	Centrifugation	Duodenum	30-300	Ensure +	5	NA	Y	[204]
Valsartan	9.791	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	9.054	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Zafirlukast	0.00521	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.00521	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Basic Drugs												
Aprepitant	0.2227	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.2114	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Atazanavir	0.0149	27	37	0.8mg/0.3ml	Centrifugation	Duodenum	10-90	Ensure +	4	23-27	Y	[214]
Carvedilol	0.369	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.4847	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Dipyridamole	0.329	5	37	135mg/25ml	Centrifugation	Duodenum	30-120	Ensure +	20	20-32	N	[224] from
	0.3171	3	37	45mg/4.5ml	Centrifugation	Duodenum	10-180	Ensure +	12	NA	Y	[150]
	0.3428	3	37	45mg/4.5ml	Centrifugation	Duodenum	10-180	Ensure +	12	NA	Y	[224]
	0.0852	24	37	NA/0.2ml	Centrifugation	Ileum	NA	NA	10	NA	N	[224]
												[181]
Itraconazole	0.0175	24	37	2mg/0.5ml	Centrifugation	Duodenum	15-90	Ensure +	5	20-30	Y	[219]
Posaconazole	0.0539	24	37	1mg/0.5ml	Centrifugation	Duodenum	NA	Ensure +	5	20-32	Y	[150]
Tadalafil	0.04212	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.04212	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
Neutral Drugs												

Drug	Solubility (mM)	Time (hours)	Temp (°C)	Solid/ Volume	Separation	HIF Sample Location	Collection Time (min)	Meal Type	HIF Sample Number	Age	Pooled	Ref
Carbamazepine	1.981	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
Danazol	0.121	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
	0.0861	24	37	NA/0.5ml	Centrifugation	Duodenum	30-300	Ensure +	5	NA	Y	[204]
	0.2819	24	37	1mg/ml	Centrifugation	Jejunum	20-60	Nutriflex	12	24-40	Y	[221]
	0.1184	4	37	1mg/1ml	Centrifugation	Duodenum	30-240	H Meal[A]	8	22-34	N	[225]
Diazepam	1.687	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
	1.796	24	37	NA/0.5ml	Centrifugation	Duodenum	30-300	Ensure +	5	NA	Y	[6]
Felodipine	1.080	24	37	1mg/ml	Centrifugation	Jejunum	20-60	Nutriflex	12	24-40	Y	[221] from
	1.078	24	37	1mg/ml	Centrifugation	Jejunum	NA	Nutriflex	NA	NA	NA	[150]
	0.4725	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[175]
	0.4777	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142] [142]
Fenofibrate	0.409	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
	0.388	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.3603	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.501	24	37	2mg/0.5ml	Centrifugation	Duodenum	15-120	Ensure +	4	19-35	Y	[222]
Griseofulvin	0.17	24	37	1mg/ml	Centrifugation	Jejunum	20-60	Nutriflex	12	24-40	Y	[221]
Prednisolone	1.69	24	37	1mg/0.3ml	Centrifugation	Duodenum	0-90	Ensure +	6	20-29	N	[147]
Probucol	0.0484	24	37	1mg/ml	Centrifugation	Jejunum	20-60	Nutriflex	12	24-40	Y	[221]
	0.07546	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]
	0.05031	24	37	2mg/1ml	Filtration	Duodenum	10-120	Ensure +	16	18-45	Y	[142]

A: Homogenised experimental meal

6.2. Materials and Methods

6.2.1. Materials

Sodium taurocholate, cholesterol, sodium chloride (NaCl), sodium oleate, ammonium formate, formic acid, potassium hydroxide (KOH), hydrochloric acid (HCl), were from Merck Life Science UK Limited, Dorset, UK. Phosphatidylcholine from soybean (PC S) was from Lipoid GmbH, Ludwigshafen, Germany. Chloroform was from Rathburn Chemical Company, Walkerburn, Scotland and sodium phosphate monobasic monohydrate ($NaH_2PO_4.H_2O$) was from Fisher Scientific, Leicestershire, UK. All acetonitrile (ACN) and methanol (MeOH) solvents were HPLC gradient (VWR). All water is ultrapure Milli-Q water.

Aprepitant and felodipine were through OrBiTo by Dr. R. Holm, Head of Preformulation, Lundbeck, Denmark. Zafirlukast was from Stratech Scientific Ltd, Ely, UK and ibuprofen was obtained from BSAF chemical company. Atazanavir and posaconazole were from Chemshuttle, Burlingame, CA, USA. Carbamazepine, carvedilol, danazol, diazepam, dipyridamole, fenofibrate, furosemide, griseofulvin, indomethacin, itraconazole, naproxen, phenytoin, piroxicam, prednisolone, probucol, tadalafil, valsartan were from Merck Chemicals Ltd, Dorset, UK.

6.2.2. Methods

6.2.2.1. Bioequivalent media stock solutions

As previously described in Section 2.3

6.2.2.2. Equilibrium Solubility Measurement

As previously described in Section 2.4

6.2.2.3. HPLC analysis

As previously described in Section 2.4.2.1

6.2.2.4. Statistical Analysis

Statistical analysis was conducted using Graphpad Prism 9 for MacOSX. Correlation analysis were performed using Datagraph 4.7.1 for MacOSX. The variable number of FaHIF measurement values do not permit a simple direct statistical comparison between the data sets. Thirteen drugs have 3 or more available FaHIF values, with seven for FeHIF and these have been compared as a group using a Wilcoxon matched pairs signed rank test, $P < 0.05$ (Two-tailed). Each drug has also been individually compared using a Mann-Whitney test, $P < 0.05$ (Two-tailed). Previous papers have highlighted that the simulated data sets [123] and Fa/FeHIF chemical compositions [126] do not follow a normal distribution and therefore non-parametric statistical comparison is appropriate. The nine media minimum and maximum solubility values (x_{min},y_{max}; x_{max},y_{min}) have been correlated using a power function ($y = ax^b$) to determine a maximum and minimum solubility boundary for each drug category, r^2 reported along with $P < 0.05$ for slope significantly non-zero.

6.3. Results and Discussion

6.3.1. Equilibrium Solubility Data Sets

One hundred and twenty nine literature Fa/FeHIF equilibrium solubility values for the measured drugs (Table 16 and Table 17) are plotted in Figure 33 and Figure 34. The data are taken from 23 published literature studies and span a single drug value, to a maximum of eight values from four studies for a single drug. The data sets are not balanced (FaHIF 84 values vs Fa9SIF 207 (23x9), FeHIF 45 vs Fe9SIF 180 (20x9)) reflecting issues associated with Fa/FeHIF availability, study drug choices and the multiple research groups performing the research.

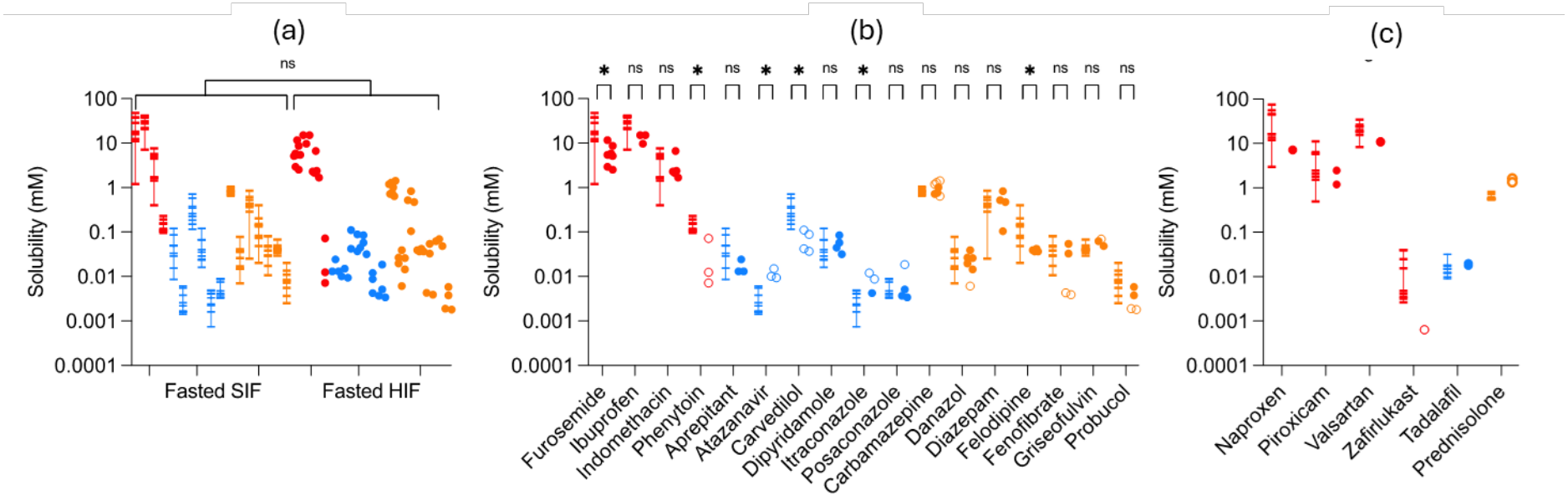


Figure 33. **Comparison plots of Fasted Equilibrium Solubility Values 9 media (Fa9SIF) and literature Fasted Human Intestinal Fluid (FaHIF)**

Note: (a) Drugs with 3 or more FaHIF solubility values. – 9 media, ● FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; ns – no significant difference between media (Wilcoxon matched pairs signed rank test, P = 0.1202 (Two-Tailed) (Pairing significantly effective P < 0.0001 (One Tailed) Spearman value = 0.9167)); drug order as per Figure 33b. (b) Drugs with 3 or more FaHIF solubility values. – 9 media, ● FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range; ns - no significant difference between media, ∗ P < 0.05. Mann-Whitney comparison individual P values, furosemide = 0.0079; ibuprofen = 0.0636; indomethacin 0.6993; phenytoin = 0.0091; aprepitant = 0.0955; atazanavir = 0.0091; carvedilol = 0.0028; dipyridamole = 0.414; itraconazole = 0.0364; posaconazole = 0.9399; carbamazepine = 0.351; danazol = 0.607; diazepam = 0.7105; felodipine = 0.0182; fenofibrate = 0.3301; griseofulvin = 0.0636; probucol = 0.0503. (c) Drugs with less than 3 FaHIF solubility values. – 9 media, ● FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range.

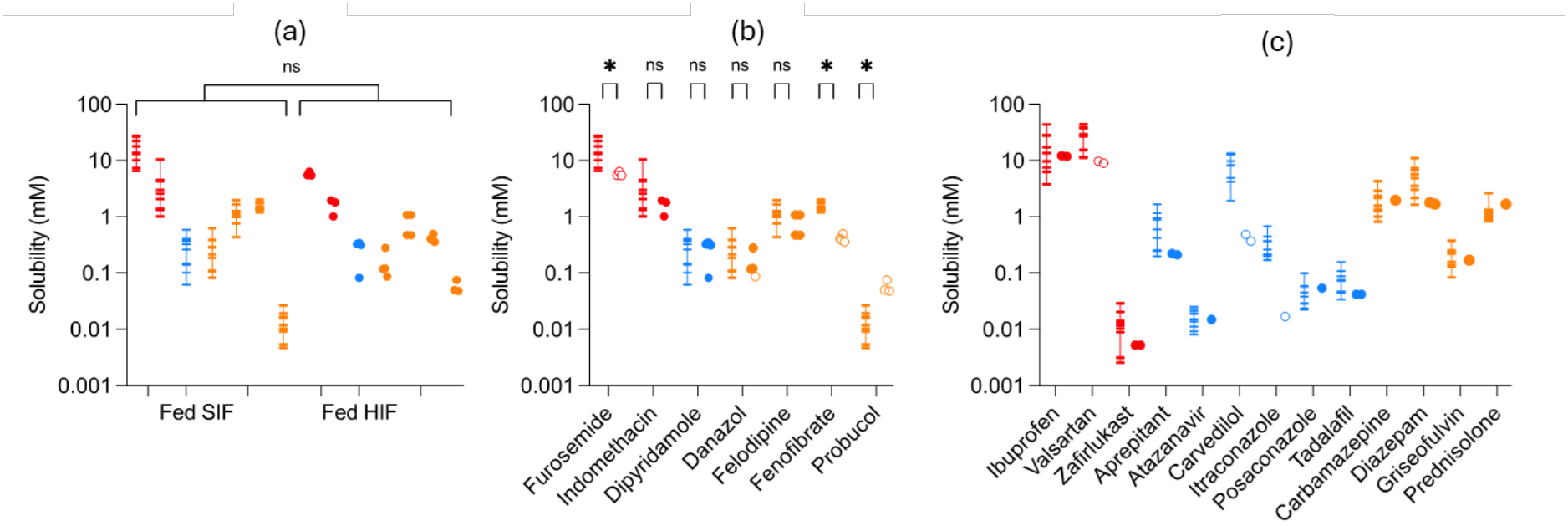


Figure 34. **Comparison plots of Fed Equilibrium Solubility Values 9 media (Fe9SIF) and literature Fed Human Intestinal Fluid (FeHIF)**

Note: (a) Drugs with 3 or more FeHIF solubility values. – 9 media, ● FeHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; ns – no significant difference between media (Wilcoxon matched pairs signed rank test, P = 0.0781 (Two-Tailed)(Pairing significantly effective P < 0.014 (One Tailed) Spearman value = 0.9643)); drug order as per Figure 34b. (b) Drugs with 3 or more FeHIF solubility values. – 9 media, ● FeHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range; ns - no significant difference between media, * P < 0.05. Mann-Whitney comparison individual P values, furosemide = 0.0091; indomethacin 0.2091; dipyridamole > 0.9999; danazol = 0.4140; felodipine = 0.2601; fenofibrate = 0.0028; probucol = 0.0091. (c) Drugs with less than 3 FeHIF solubility values. – 9 media, ● FaHIF; red = acidic drugs, blue = basic drugs, orange = neutral drugs; closed symbols value lies within 9 media solubility range, open symbols value lies outside range.

6.3.2. Human Intestinal Fluid Experimental Protocols

The Fa/FeHIF collection and solubility measurement protocols vary (Table 16 and Table 17) with potential to influence the SIF vs HIF comparison. The duodenum and jejunum predominate as a sampling location and HIF compositional data indicates minimal differences between these sites [149], although FeHIF comparisons are limited. Subject ages range from 18 to 49 in the fasted and 45 in the fed, with an average study span of 16 and 19 years respectively. Age effects on HIF solubility have been investigated [212] and although samples exhibited a high interindividual variability, specific age-dependency was not observed. The study utilised to calculate Fa/Fe9SIF[159], sampled from the duodenum with an age range from 18-31, parameters consistent with the Fa/FeHIF protocols.

The average subject number per HIF measurement is 10 in the fasted state and 11 in the fed state, with a range of 4 to 20 and sample pooling in 63% of FaHIF and 74% of FeHIF measurements. Where Fa/FeHIF samples are not pooled there will be solubility variation due to inter and intra individual compositional variability[126]. Pooling will mitigate variability dependent upon number of samples, but pools will have an unknown composition. Fa/Fe9SIF is based on 20 volunteers and 324 samples comparable to the Fa/FeHIF protocols, but due to the variability unlikely to be identical especially for un-pooled and low number pooled measurements. Seventy nine percent of the fed state protocols use Ensure Plus™ as a standard meal with a mean collection time of 110 minutes starting on average 10 minute after Ensure administration. This is comparable to the study utilised to calculate Fe9SIF[159], although differences in sampling duration (90 minutes vs 270) for some studies may have an impact[207].

Solubility measurement protocols are consistent with incubation at 37°C, equilibration time of ≥24 hours and separation of undissolved drug prior to analysis. In one case, room temperature was applied, which will have a minor impact on solubility[226]. Studies indicate that for slowly dissolving drugs to attain equilibrium solubility requires twelve hours[160] and only 3 hours for soluble drugs. One study

utilised a 3 hour incubation time and it is noticeable that the poorly soluble drugs (phenytoin and itraconazole) exhibit low solubility compared with Fa/Fe9SIF, whilst the soluble (furosemide and dipyridamole) do not. Drug solid form has not been uniformly assessed, which could impact solubility; for atazanavir for example only the crystalline equilibrium solubility values have been utilised.

6.3.3. Summary

Since the literature Fa/FeHIF solubility data arise from 23 different studies it is inevitable that there will not be absolute consistency between experimental protocols. This will produce variability that can impact the comparison and two main sources can be identified the Fa/FeHIF sampling protocol and the solubility determination in the sampled fluid.

The Fa/Fe9SIF media were calculated to cover 95% [126] of the compositional variability of a Fa/FeHIF data set taken from 20 volunteers [159]. Literature information on the composition of HIF samples and the impact of sample pooling is limited as well as the potential impacts of changing physiological factors such as sampling site and volunteer status. This issue is further discussed in Section 6.3.4.1 for a Fa/FeHIF study which includes compositional data. The fasted state, as a resting state is likely to exhibit greater compositional consistency than the fed state which will be more dynamic as digestion and intestinal transit occurs [198]. With the additional complication for the fed state of the nature of the meal ingested. The solubility determination protocol is generally consistent as discussed above.

The analysis indicates that although Fa/Fe9SIF were calculated to cover 95% of Fa/FeHIF compositional space, the solubility comparison limits should be relaxed to allow for the multiple issues discussed above. Irrespective of the comparison and variability problems, realistically the approach applied is all that is possible due to the inherent issues associated with the literature results.

6.3.4. Comparison of Solubility Data Sets

Previous SIF solubilities are not normally distributed[123] therefore non-parametric statistical comparison is required. There are seventeen fasted drugs and seven fed with three or more Fa/FeHIF values; comparison of prandial groups (Wilcoxon matched by drug pairs test) calculates no significant solubility difference between FaHIF and Fa9SIF or between FeHIF and Fe9SIF (Figure 33a and Figure 34a). When drugs are compared individually (Mann-Whitney test) there is no significant difference for 11 out of 17 drugs between FaHIF and Fa9SIF (Figure 33b) and for 4 out of 7 drugs between FeHIF and Fe9SIF (Figure 34b). The fasted felodipine difference is due to the narrow FaHIF solubility distribution a result possibly due to FaHIF pooling. Furosemide displays a similar behaviour but this cannot be attributed to pooling.

For felodipine and furosemide the statistical difference is not significant, FaHIF values within Fa9SIF range, based on this study's aim. Therefore, for fasted 76% (82% if the phenytoin result is excluded due to the lower equilibration time) and in the fed 57% of the drugs with ≥3 HIF solubility values there is no individual significant solubility difference between Fa/Fe9SIF and Fa/FeHIF. Comparison of individual FaHIF solubility values with the Fa9SIF range (Figure 33b/c) indicates that 68% are within the boundaries and in the fed state the value is 64% (Figure 34b/c). One study correlated ten poorly soluble drugs in three different FaSIF media and where a comparison to FaHIF is presented 48% were within the range[176]. Fa9SIF agreement is higher, expected based on the range coverage compared to individual FaSIF media, although the difference between the studies and drugs examined impacts this comparison.

6.3.4.1. Impact of composition on solubility behaviour

Comparison of solubility behaviour determined in fasted [160] and fed [130] state DoE studies reinforces the correlation discussed above. Fa9SIF media composition has minimal impact on carbamazepine solubility [203] a feature that is present for FaHIF

solubility values from five studies (Figure 33b). One study [142] analysed HIF pooled from 16 volunteers for pH, bile salt and phospholipid, three of the five Fa/Fe9SIF components or dimensions, which can be compared with Fa/Fe9SIF values[126]. The pooled fasted bile salt (FaHIF 3.52mM vs Fa9SIF 1.06-11.45mM)/phospholipid (0.16mM vs 0.19-2.48mM) ratio is low compared to Fa9SIF (Figure 35a), whilst the bile salt/pH (6.83 vs 5.72-8.04) ratio is in the centre. The pooled fed state pH (FeHIF 5.96 vs Fe9SIF 5.86-6.59)/bile salt (8.91mM vs 4.94-19.04mM) ratio (Figure 35b) is low when compared to Fe9SIF range whilst the bile salt/phospholipid (3.72mM vs 2.07-7.94mM) ratio is in the centre. For acidic drugs pH is the major solubility driver[130, 160, 203, 209] hence in the fasted comparisons ibuprofen and valsartan are equivalent, zafirlukast requires bile salt and phospholipid solubilisation[160], which in the pooled FaHIF are low and could be linked to the low solubility value. In the FeHIF pool this is reversed where valsartan solubility is low due to the low pH but zafirlukast is equivalent due to the "normal" bile salt phospholipid concentrations. Probucol requires monoglyceride for solubility [130] a component not in Fe9SIF but present in the pooled FeHIF [159], potentially explaining the higher solubility.

These examples illustrate the issue of reconciling different drugs' solubility behaviour in media of defined against unknown composition. The results indicate that increasing the number of HIF values increases compositional coverage and provides a greater chance of agreement with Fa/Fe9SIF, multiple drugs have solubility values outside the Fa/Fe9SIF range but this is not statistically significant (Figure 33 and Figure 34). Highlighting that the single value comparison is a stringent test and multiple value comparisons provide greater coverage. This implies that a larger HIF composition data set is required to improve the analysis using more or different dimensions[126], and that HIF solubility measurement should be linked to chemical composition[142]. This latter modification would permit a systematic comparison of HIF and SIF solubility.

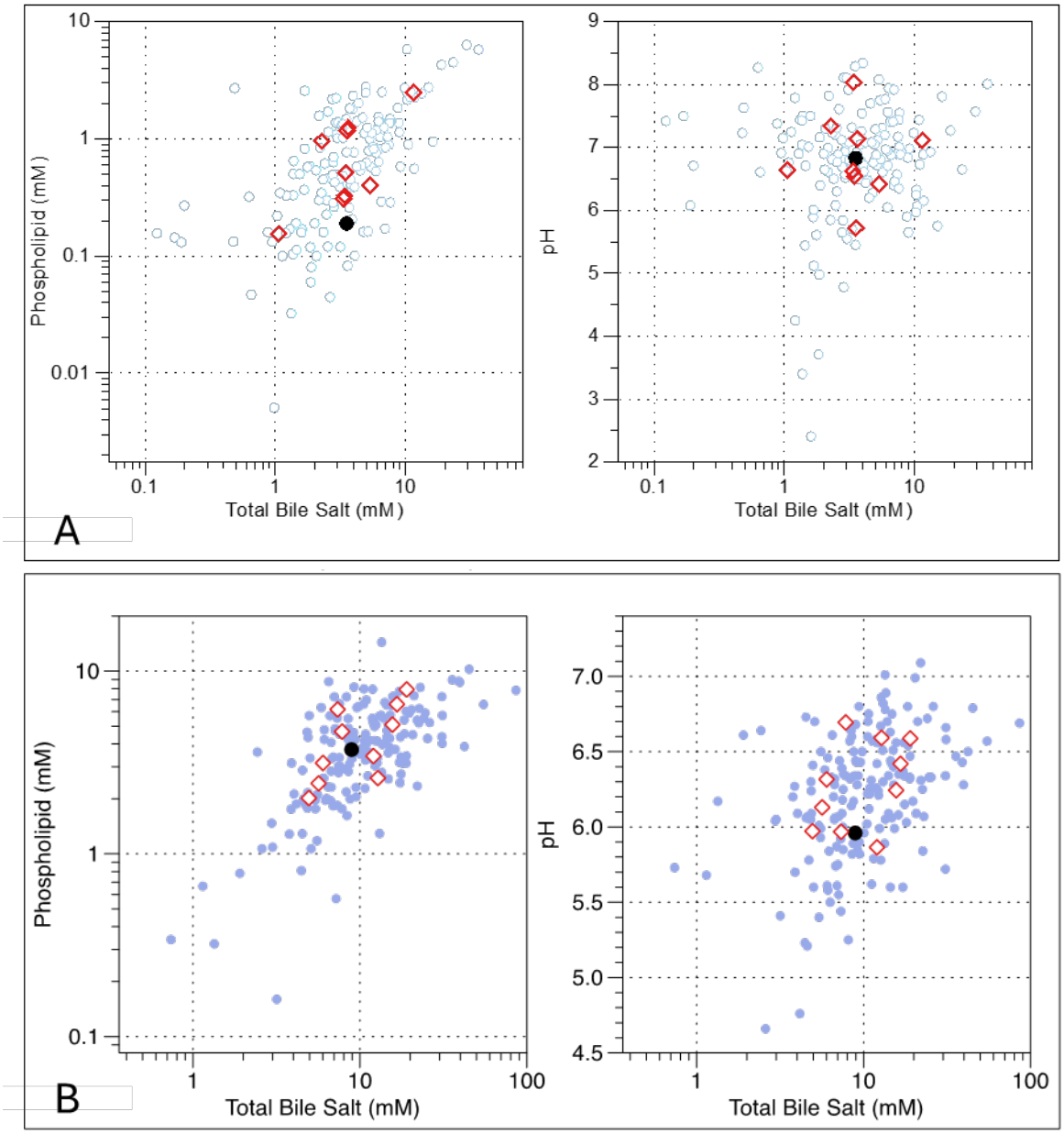


Figure 35. **Compositional comparison Fa/Fe9SIF and sampled pooled FaHIF**

Note: (a) Dahlgren Pooled FaHIF Composition vs FaHIF Data Set and Fa9SIF Composition. ○ Bile salt, phospholipid and pH individual sample values from Pyper [126]. Fa9SIF ◇ nine media points; ● Dahlgren[142] pooled FaHIF values. (b) Dahlgren Pooled FeHIF Composition vs FeHIF Data Set and Fe9SIF Composition. ● Bile salt, phospholipid and pH individual sample values from Pyper [126]. Fe9SIF ◇ nine media points; ● Dahlgren[142] pooled FeHIF values.

6.3.5. Solubility Correlation Boundary

To extend the literature Fa/FeHIF comparison for the drugs measured using Fa/Fe9SIF, upper and lower correlation boundaries have been calculated based on the minimum and maximum solubility values (xmin,ymax; xmax,ymin where min or max

represents the Fa/Fe9SIF minimum and maximum solubilities) and plotted graphically (Figure 36a). The acidic and basic drug correlations are statistically significant and for neutral drugs in the fed state but not the fasted, this is not critical since the relationship defines a boundary with a span equal to the average solubility range for each drug category. The boundaries shape reflects drug category solubility behaviour previously determined by DoE studies[130, 160]. Acidic drug solubility is pH driven and the similarity of pH ranges between Fa9SIF (5.72 – 8.04) and Fe9SIF (5.97 – 6.59) leads to contiguous boundaries with fed (lower pH range) inside the fasted. Basic and neutral drug solubility is driven by pH and total amphiphile content (pH x TAC) and the difference between Fa9SIF (15.1 – 122.4) and Fe9SIF (109.1 – 493.1) is reflected in the boundaries. The boundary changes between fasted and fed states for these drug classes is indicative of solubility changes between fasted and fed states and the presence of a food effect, see next section.

An additional literature[150] Fa/FeSIF vs Fa/FeHIF solubility data set of 66 values for 25 drugs has been plotted with the boundaries (Figure 36b) and 95% are inside. This is a first exploration of this relationship and reinforces the statistical conclusion that Fa/Fe9SIF provide an *in vitro* in vivo solubility correlation, but should be treated with caution. A wide enough boundary will accommodate any data, especially if centred on the equivalence line around which correlation is unavoidable. In addition, the boundary is based on the study drugs which may not be a representative sample.

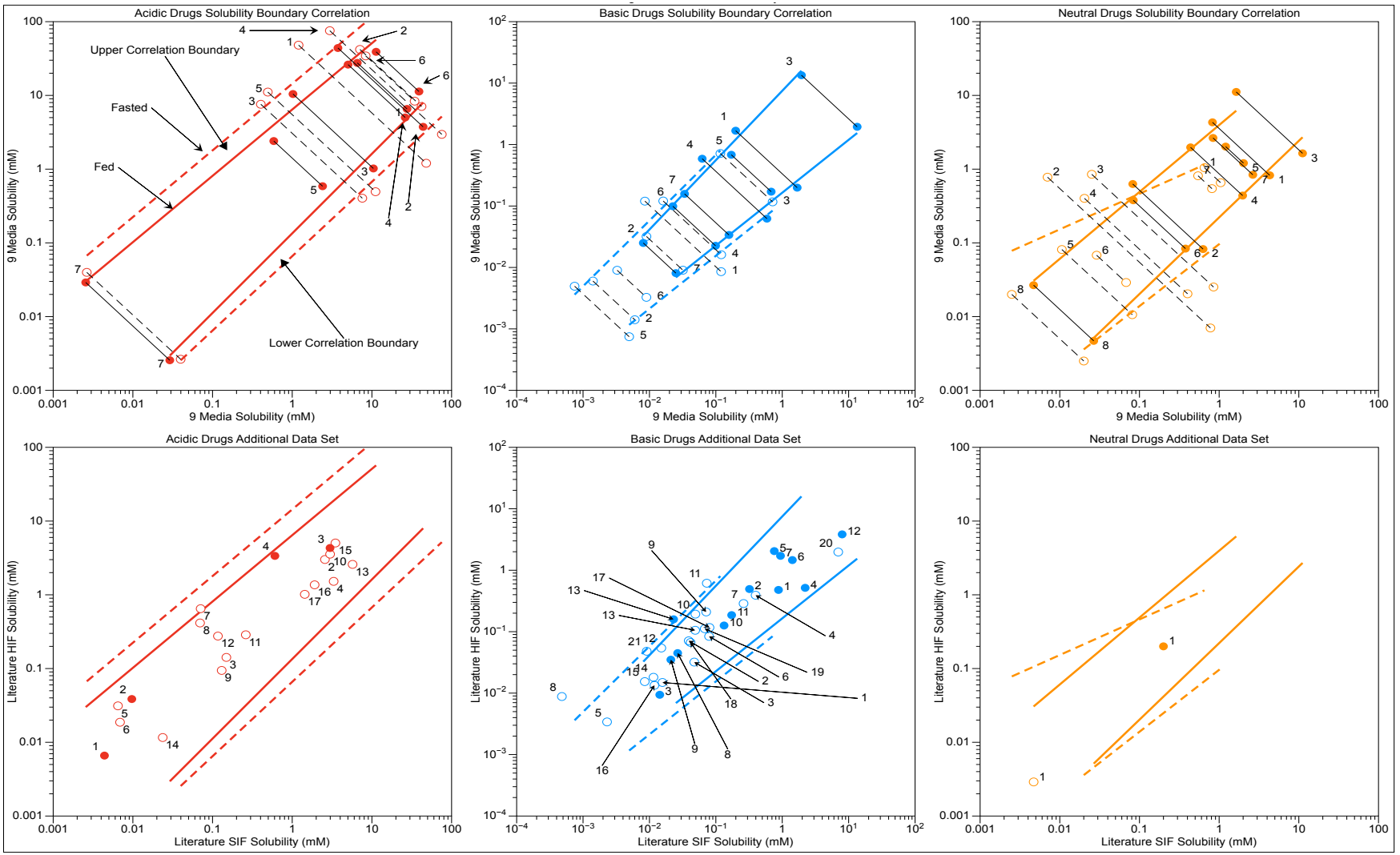


Figure 36. **Solubility Boundary Correlation; (a) Solubility boundary correlation – Upper Panel; (b) Additional literature data comparison**

Note: Figure 36a. Solubility boundary correlation – Upper Panel. Acidic, basic and neutral, fasted and fed, upper and lower solubility correlation boundaries based on the minimum and maximum solubility for individual drugs (see numbers) in each Fa/Fe9SIF state plotted as x_{min},y_{max} and x_{max},y_{min} (fasted open symbol, fed closed symbol, fasted drug points connected by dashed black line, fed solid black line); best fitting power correlation line ($y = A\ x^B$) (fasted – dashed coloured line; fed – solid coloured line). Acidic Drugs 1- Furosemide, 2-Ibuprofen, 3-Indomethacin, 4-Naproxen, 5-Piroxicam, 6-Valsartan, 7-Zafirlukast. Basic Drugs 1-Aprepitant, 2-Atazanavir, 3-Carvedilol, 4-Dipyridamole, 5-Itraconazole, 6-Posaconazole, 7-Tadalafil. Neutral Drugs 1-Carbamazepine, 2-Danazol, 3-Diazepam, 4-Felodipine, 5-Fenofibrate, 6-Griseofulvin, 7-Prednisolone, 8-Probucol.
Acidic Drugs
Lower Correlation Boundary: Fasted y = 0.066013*x^1.009, R^2 = 0.9122, P = 0.0008; Fed y = 0.13456*x^1.0792, R^2 = 0.9707, P < 0.0001.
Upper Correlation Boundary: Fasted y = 14.389*x^0.90412, R^2 = 0.9122, P = 0.0008; Fed y=6.4331*x^0.89946, R^2 = 0.8995, P < 0.0001.
Basic Drugs
Lower Correlation Boundary: Fasted y = 0.11225*x^0.86083, R^2 = 0.9200, P = 0.0006; Fed y= 0.16457*x^0.8606, R^2 = 0.9716, P = < 0.0001.
Upper Correlation Boundary: Fasted y = 7.9449*x^1.0687, R^2 = 0.9200, P = 0.0006; Fed y= 7.4946*x^1.1289, R^2 = 0.9716, P < 0.0001.
Neutral Drugs
Lower Correlation Boundary: Fasted y = 0.096135*x^0.84039, R^2 = 0.4058, P = 0.0894; Fed y= 0.22133*x^1.0385, R^2 = 0.9420, P < 0.0001.
Upper Correlation Boundary: Fasted y = 1.41*x^0.48289, R^2 = 0.4058, P = 0.0894; Fed y = 3.9606*x^0.90705, R^2 = 0.9420, P < 0.0001.
Figure 36b. Additional literature data comparison.
Acidic, basic and neutral, fasted and fed, upper and lower solubility correlation boundaries based on the minimum and maximum solubility for individual drugs in each Fa/Fe9SIF state Fasted open symbol, fed closed symbol, Fasted – dashed coloured line; Fed – solid coloured line. Additional solubility data from [150]. Fasted – open symbols, Fed – closed symbols. Acidic Drugs Fasted 1-Atovaquone, 2-Diclofenac, 3-Diethylstilbestrol, 4-Flufenamic acid, 5&6-Glibenclamide, 7, 8&9-Glipizide, 10-Hydrochlorothiazide, 11-Irbesartan, 12-Nimesulide, 13-Probenecid, 14-Rimonabant, 15&16-Sulfasalazine, 17-Warfarin. Acidic Drug Fed 1-Glibenclamide, 2-Glipizide, 3-Hydrochlorothiazide, 4-Sulfasalazine. Basic Drugs Fasted 1&2-AZD0865, 3-Cinnarizine, 4-Darunavir, 5-Etravirine, 6-Indinavir, 7-Irbesartan, 8-Itraconazole, 9,10,11,12&13-Ketoconazole, 14,15&16-Loviride, 17,18&19-Nifedipine, 20-Quinidine, 21-Ritonavir. Basic Drug Fed 1-Cinnarizine, 2-Darunavir, 3-Etravirine, 4-Indinavir, 5,6&7-Ketoconazole, 8&9-Loviride, 10&11-Nifedipine, 12-Quinidine, 13-Ritonavir. Neutral Drugs Fasted 1-Cyclosporine. Neutral Drugs Fed 1-Cyclosporine.

6.3.6. Potential Biopharmaceutical Application

The DCS [174] applies a single poorly characterised Fa/FeHIF or Fa/FeSIF solubility measurement to evaluate a drug's potential biopharmaceutical performance. Fa/Fe9SIF is an advance by providing a bioequivalent solubility range (see above) linked to an intestinal solubility population distribution, which can be applied to provide DCS[161, 201] boundary limits. Absorption depends on the solubility, intestinal permeability interplay (other issues e.g. first-pass metabolism are not considered in this paper), which along with intestinal transit time and surface area can be utilised to calculate a Solubility Limited Absorbable Dose (SLAD)[174]. The utility of a bioequivalent Fa/Fe9SIF solubility range can be visualised by calculating the Dose/SLAD ratio and plotting against the intestinal solubility population distribution (Figure 37).

Dose/SLAD < 1, indicates that intestinal equilibrium solubility, permeability and transit time is sufficient to permit complete absorption and the highest value for ibuprofen provides a >10 fold solubility excess or safety factor. Dose/SLAD > 1, indicates that intestinal solubility, permeability and transit time is not sufficient to permit complete absorption and for griseofulvin that the maximum solubility deficit is >10 fold. This provides a performance level, supersaturated concentration and time relationship, for formulation strategies, for example amorphous systems [227], to ensure complete absorption. The Fa9SIF, Fe9SIF griseofulvin Dose/SLAD curves, indicate that there is a fed state induced solubility difference and since the curves do not overlap is detecting *in vitro* the known griseofulvin food effect [183]. Other drugs also display this phenomenon (Figure 36a, e.g dipyridamole) indicating that this result is worthy of further examination for the *in vitro* detection of solubility based food effects.

Fa/Fe9SIF display structured solubility distributions that permit identification of the minimum and maximum solubility media for the drug categories [203, 209]. This permits a pick-n-mix, drug development stage or requirement based approach for intestinal solubility measurement [206]. A total intestinal solubility range screen can be

assessed with two measurements, both prandial states with four, providing assessment of potential food effect and eighteen to provide the full assessment.

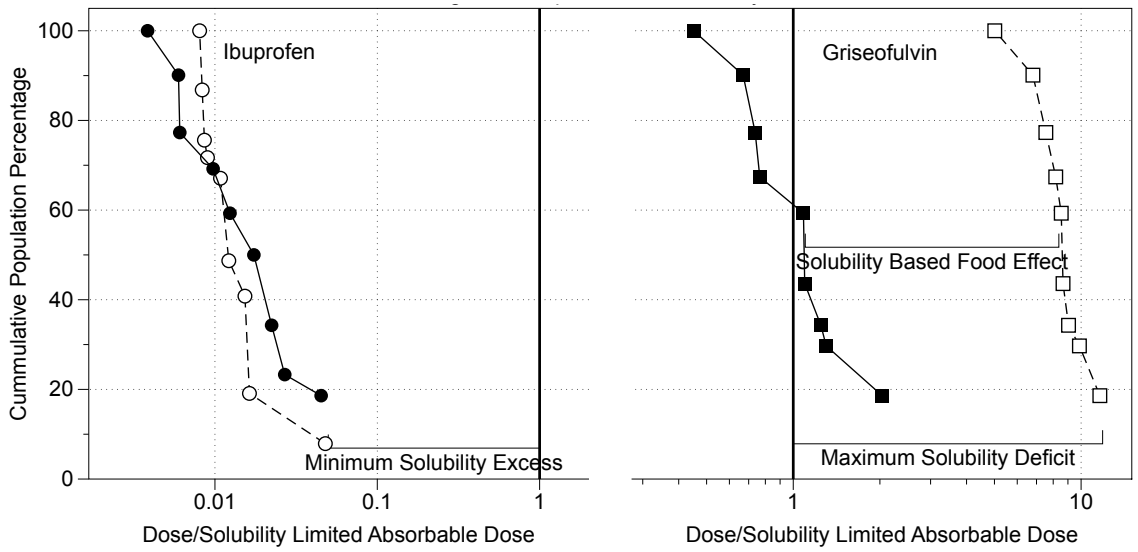


Figure 37. **Biopharmaceutical analysis**

Note: Ibuprofen Fasted ○; Fed ● Dose = 400mg: Griseofulvin Fasted □; Fed ■ Dose = 500mg.

6.4. Conclusions

The *in vitro* in vivo comparison of intestinal solubility is in principle simple but confounded by multiple factors associated with HIF's natural variability and limited availability. Twenty three drugs are not a comprehensive or structured sample and arises due to published study choices, which limits comparison. This could be ameliorated by targeting additional drugs with multiple Fa/FeHIF (≥3) measurements or optimally a compositional assessment of Fa/FeHIF prior to solubility measurement.

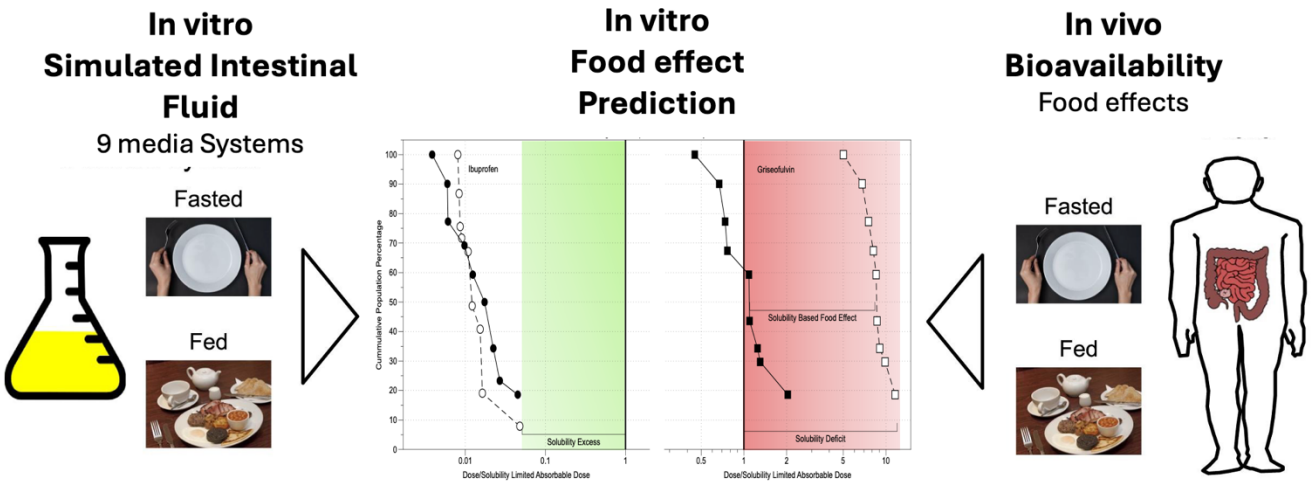
Statistical comparison does not detect a significant solubility difference between Fa9SIF and FaHIF or Fe9SIF and FeHIF data sets. The result indicates that the Fa/Fe9SIF solubility range can be considered bioequivalent to Fa/FeHIF. A novel comparison based on solubility boundaries encompasses 95% of an additional solubility data set, further reinforcing the statistical conclusion of *in vitro* in vivo correlation. Solubility differences and behaviour can be linked to SIF DoE study results and the influence of media components, indicating that further intestinal fluid composition assessment can refine

the approach delivering the potential to measure *in vitro* intestinal solubility in multiple population and patient groups or species.

An *in vitro* bioequivalent solubility range measurement incorporating population distribution information[161, 201] expands DCS[174] approaches to biopharmaceutical performance assessment. A novel graphical analysis utilising the administered dose divided by the solubility limited absorbable dose permits the calculation of drug and dose related solubility safety margins, formulation performance requirements and potential solubility based food effects. Since equilibrium solubility [138] is a key parameter controlling oral absorption an *in vitro* bioequivalent measurement can be applied to refine PBPK[228] and in silico modelling with potential to generate individual or disease related intestinal solubility profiles and reduce in vivo testing. The Fa/Fe9SIF system is therefore worthy of further investigation with linkage of system results to in vivo performance a key next stage and may also represent a methodology applicable to other multicomponent biological fluids where no single component is responsible for performance.

Chapter 7

Using Biorelevant *in Vitro* Fasted/Fed Solubility Systems to Support Food Effect Prediction



This chapter aims to develop a new tool for predicting food effects on drug absorption using simple solubility measurements and SLAD calculations, and correlate findings with in vivo bioavailability and food effect predictions from literature.

A manuscript with the data presented in this chapter is in preparation for publication.

7.1. Introduction

7.1.1. Fasted and Fed States

When examining drugs in either SIF or HIF, it is crucial to analyse the fasted and fed states separately due to variations in gastrointestinal conditions and intestinal fluid characteristics between each state.

As previously discussed in Chapter 1, the fasted state, achieved typically through overnight fasting, is marked by the absence of food in the gastrointestinal tract. The composition of fasted human intestinal fluid is therefore regarded as representative of gastrointestinal physiology without the influence of food and digestion products. The fed state is achieved after food consumption and comprises a complex system characterised by unique gastrointestinal fluid composition and considerable variability due to intersubject variations and food consumed [229]. Drug absorption during the fed state can be affected by various factors, including the nature of the meal (solid or liquid), its caloric content, nutrient composition (such as high-fat, high-protein, or high-carbohydrate meals), meal temperature, fluid intake and volume [53, 170, 171, 229].

7.1.2. Food Effects on Drug Absorption

The conditions of the fed state and the postprandial changes in gastrointestinal physiological variables (such as increased bile flow, pH variations, and the presence of digestion products) can lead to either an increase (positive) or a decrease (negative) in overall bioavailability [171, 229]. Changes in a drug's bioavailability from the fasted to the fed state are referred to as food effects. Positive food effects are associated with enhanced solubility and dissolution of drugs, delayed gastric emptying times, inhibition of metabolic enzymes and efflux transporters in the first-pass route, and alterations in luminal metabolism, resulting in increased drug absorption. Conversely, negative food effects stem from drug degradation due to gastric acid secretion, direct physical or chemical interactions with the drugs, and increased intestinal motility [167, 171, 172, 230, 231]. Consequently, the co-administration of drugs and food can significantly

influence both the rate and extent of drug absorption, thereby impacting therapeutic performance [232].

As stated in Chapter 1, variations between the fasted and fed states are anticipated, therefore it is crucial to define what qualifies as a significant food effect. The FDA [233] specifies that if the 90% confidence intervals for the ratio of population geometric means for Cmax and AUC between fed and fasted states fall outside the 80–125% bioequivalence limits established for the fasted state, a food effect with potential clinical significance is confirmed and warrants consideration.

7.1.3. *In Vitro* Prediction of Food Effects

In the initial stages of drug development, investigating food effects can be challenging due to the limited availability of compound for testing [234]. In vivo studies related to food effects typically involve animals or human volunteers, rendering them costly, resource-intensive and requiring significant time and effort. While *in vitro* prediction models based on permeability and solubility may not suffice for regulatory decisions, they still offer valuable insights that are useful for screening potential food effects in early drug development without great investment [235].

As stated in Chapter 1, the BCS [236] links a drug´s *in vitro* solubility and permeability with its in vivo bioavailability and it is often used as an *in vitro* prediction tool. BCS Class I drugs typically exhibit higher solubility and no absorption issues, thus are considered less impacted by food. BCS Class II comprises drugs with low solubility values, making them more prone to positive food effects. Drugs categorised as BCS Class III, with low permeability values, are likely to experience negative food effects, while BCS Class IV compounds are harder to predict due to their diverse physicochemical characteristics [237, 238]. The Biopharmaceutics Drug Disposition Classification System (BDDCS) [238] uses the BCS as the base for their food effects prediction and hypothesises that drug-transporter interactions are a primary mechanism for food effects, with high-fat meals potentially inhibiting drug transporters. BCS Class II compounds may see increased bioavailability due to efflux transporter inhibition and

solubilisation, while Class III compounds may experience decreased bioavailability due to uptake transporter inhibition. Predicting food effects for Class IV compounds was still challenging due to the complexity of interacting factors. However, it is suggested that high-fat meals may increase bioavailability for these compounds by enhancing solubilisation and inhibiting efflux transporters.

The DCS [28] extends the solubility concept introduced by the BCS by incorporating the use of FaSSIF media to assess drug solubility. It builds upon BCS Class II and introduces two subcategories: Class IIa (for compounds with absorption limited by dissolution) and Class IIb (for compounds with absorption limited by solubility). Consistent with BCS predictions, drugs classified as solubility limited in DCS Class IIb and Class IV exhibit the highest propensity for food effects [239].

Applying these solubility/permeability approaches to predict food effects can be challenging since numerous factors that influence the pharmacokinetic profile of a drug (such as Cmax) are too complex to be accurately captured by such simplistic methods [231, 239]. However, the advantage of these approaches lies in their ability to predict the direction of a food effect (positive, negative, or none), which can be easily determined and useful for screening purposes [231, 239, 240].

7.1.4. *In Vitro* Prediction of Food Effects Using Biorelevant Fasted and Fed Media Systems

Solubility assessment in simulated biorelevant media commonly serves as an initial step for predicting food effects especially when poorly water-soluble drugs are identified [234, 241, 242]. Selecting biorelevant simulated fluids for measuring solubility in both states ensures a stronger correlation with HIF values, thus providing more accurate food effect predictions. Differences in fasted and fed solubility only translate in significant differences in bioavailability when a drug's absorption is incomplete, usually due to low solubility and/or slow dissolution [239]. Therefore, these approaches

might be more useful for drugs with poor solubility, usually BCS classII and IV, that have more propensity to present positive food effects.

In this study, nine biorelevant SIF recipes representative of more than 95% of the component variation of intestinal media for the fasted [243] and fed states [244] (Chapter 3), were used to determine the solubility ranges for a group of twenty three examples of neutral, acidic and basic drugs (See Chapter 2). The media recipes originated from sampled Fa/FeHIF [89] and are more likely to be bioequivalent. The fed state presents a limitation since in the original collection study it was achieved by the administration of the liquid feed Ensure Plus™[89].

Our aim was to apply and compare these biorelevant Fa/FeSIF systems, which guarantee a stronger correlation with in vivo conditions, to create a new tool for predicting the direction of food effects using simple solubility measurements and SLAD calculations. All findings were compared and correlated with in vivo bioavailability and food effect predictions found in the literature.

7.2. Materials and Methods

7.2.1. Materials

Sodium taurocholate, cholesterol, sodium oleate, sodium chloride (NaCl), ammonium formate, potassium hydroxide, hydrochloric acid (HCl) and formic acid were purchased from Merck Chemicals Ltd. Lecithin S PC (phosphatidylcholine from Soybean "98%") was purchased from Lipoid®Germany. Chloroform was obtained from Rathburn Chemical® and FeSSIF-v2 media from Biorelevant.com Ltd. Sodium phosphate monobasic monohydrate ($NaH2PO4{\cdot}H_2O$) was bought from Fisher Scientific.

The active pharmaceutical ingredients Furosemide, Naproxen, Piroxicam, Mefenamic acid, Phenytoin, Zafirlukast, Indomethacin, Dipyridamole, Carvedilol, Aprepitant, Tadalafil, Posaconazole, Atazanavir, Acyclovir, Griseofulvin, carbamazepine, fenofibrate and probucol were purchased from Merck Chemicals Ltd. Ibuprofen was purchase from BSAF chemical company, paracetamol was obtained from Mallinckrodt Pharmaceuticals and felodipine was acquired from USbiological Life Sciences.

The water was ultrapure Milli-Q water and the solvents Methanol (VWR®, UK) and Acetonitrile (VWR®, UK) were HPLC grade.

7.2.2. Methods

7.2.2.1. Stock media solutions for fasted and fed solubility experiments

7.2.2.1.1. Biorelevant media stock solutions

As previously described in Section 2.3

7.2.2.2. Equilibrium solubility measurement

As previously described in Section 2.4

7.2.2.3. HPLC analysis

As previously described in Section 2.4.2.1

7.2.2.4. Data analysis

Data analysis and comparison was conducted using Graphpad Prism 9 and DataGraph for MacOSX.

7.3. Results and Discussion

7.3.1. Solubility Comparison

The equilibrium solubility measured using the nine biorelevant SIF recipes for the fasted and fed states are displayed and compared in the figures below (Figure 38 to Figure 40). Since the media recipes were formulated based on HIF composition [89], the solubility ranges found for both states are biorelevant (see previous section). These multipoint systems allow to study the impact of media variation on drug solubility, offering more information regarding drug behaviour than single measurement approaches [129, 161, 243-246]. In order to compare the fasted and fed state solubility values, a non-parametric statistical comparison (Mann-Whitney test) was performed, and significant differences were considered when $p<0.05$.

In Figure 38, the solubility ranges of acidic drugs in both fasted and fed sates are illustrated. For most acidic drugs, there were no statistical differences found between the fasted and fed states. Only piroxicam and phenytoin, two weak acids, were the exception. Previous studies have reported that the solubility of acidic drugs is primarily influenced by pH, with media composition having a greater impact on drugs with higher pKa values. Generally, solubility tends to increase with higher pH values.

In this study, the pH of both systems differs. The pH in the fasted state ranges from 5.72 to 8.04 (Δ = 1.62), while in the fed state, it ranges from 5.97 to 6.59 (Δ = 0.62). However, it appears that this difference did not significantly affect the solubility of most acidic drugs, as they behaved consistently in both states. Piroxicam (pKa = 6.3) and phenytoin (pKa = 8.33) have higher pKa values and seem to be more affected by the changes in media between states. Piroxicam exhibits lower solubility values and a narrower solubility range in the fed state compared to the fasted state. This could be attributed to the pH difference between the two states, as the fasted state has higher pH values and a wider pH range than the fed state. Phenytoin shows an increase in solubility in the fed state, consistent with previous studies [129, 246] that suggest drugs with higher pKa values are more sensitive to variations in media composition, resulting in higher solubility in the fed state in this case. Although pH was considered to have a twenty times greater impact than any media amphiphilic component on the solubility of

acidic drugs [121, 122], the differences in pH values between the fasted and fed states did not lead to a significant shift in solubility for the examples analysed, as indicated by the direct comparison of the fasted and fed systems.

Figure 39 and Figure 40 display the solubility ranges of basic and neutral drugs, respectively. In contrast to acidic drugs, the majority of neutral and basic drugs exhibit significant differences between the fasted and fed states, with probucol being the only exception. Previous studies employing these media systems in both states [121, 122] have determined that the solubility of basic drugs is still influenced by pH, but to a lesser extent than acidic drugs, and that the total amphiphile content of the media is the primary factor affecting solubility for both basic and neutral drugs. The total amphiphile concentration (TAC) comprises the sum of all component concentrations: bile salts, fatty acids, phospholipids, and cholesterol. In the fasted state, it ranges from 2.27 to 17.19 mM, and in the fed state, from 18.41 to 74.83 mM. Given the considerably higher TAC in the fed state, it is expected that for most of these drugs, solubility is increased in the fed state, as evident in Figure 39 and Figure 40.

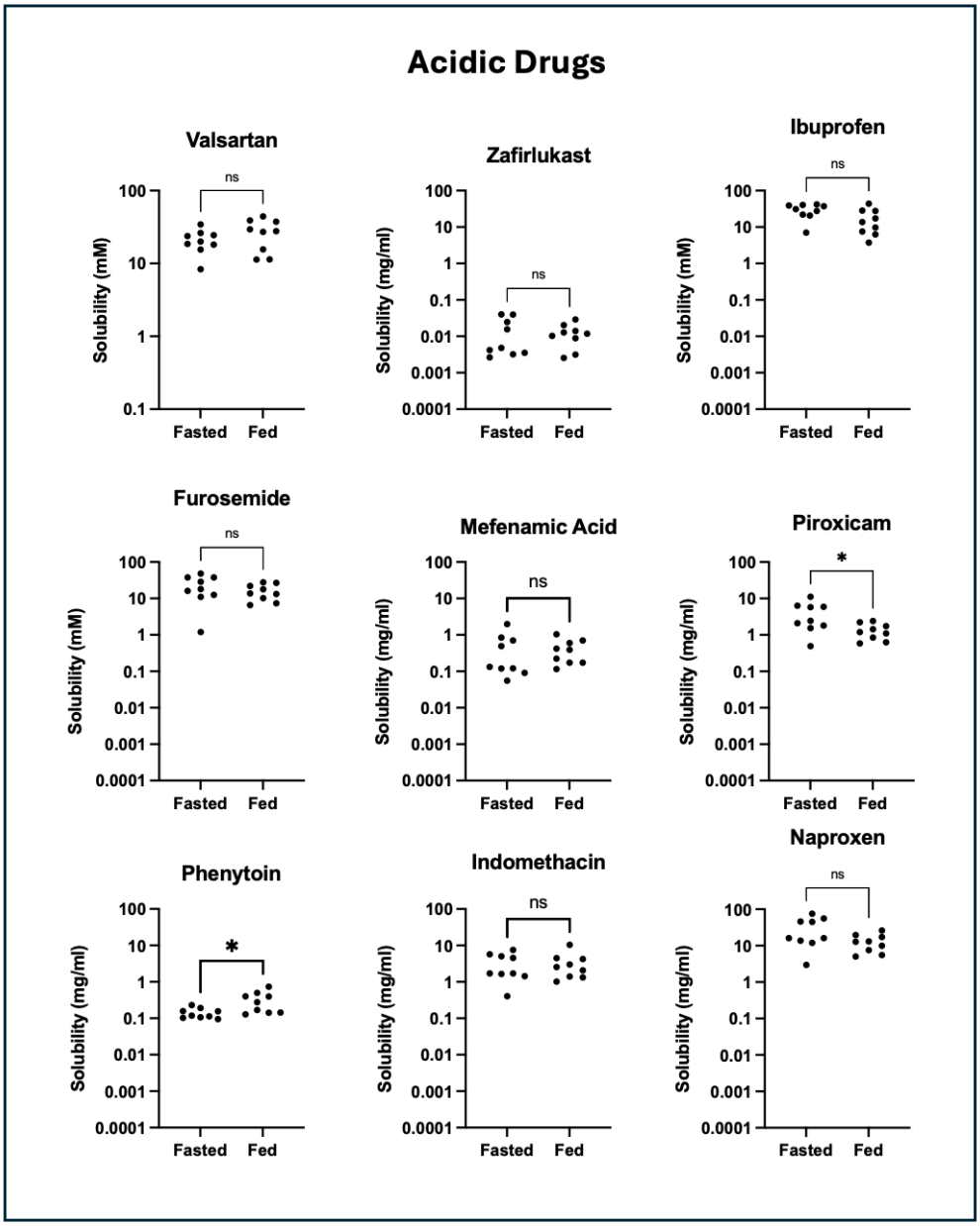


Figure 38. **Solubility comparison fasted vs fed state for acidic drugs**

Note: Mann-Whitney Statistical test applied. ns= non-significant; * p≤0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001.

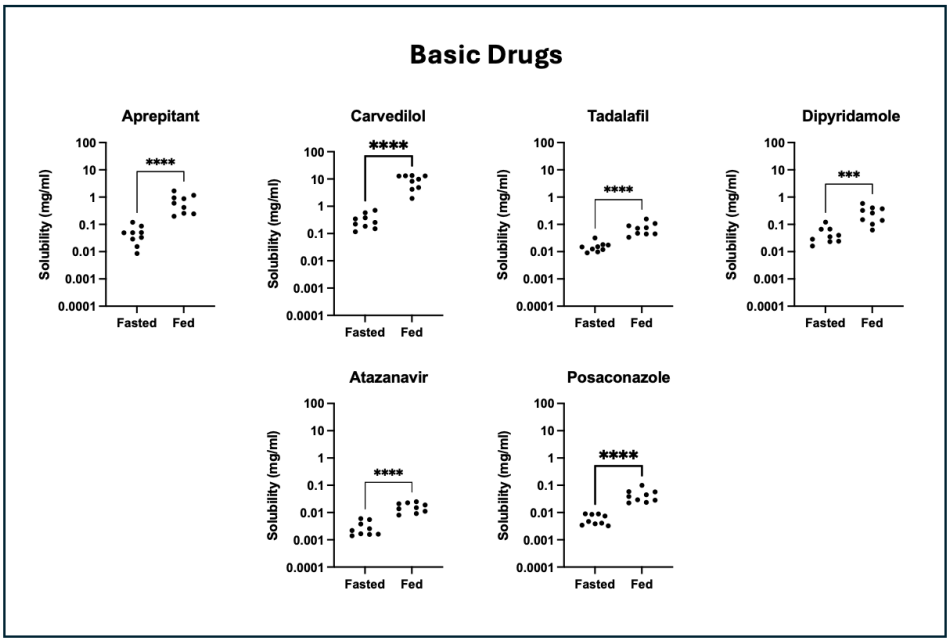


Figure 39. **Solubility comparison fasted vs fed state for basic drugs**

Note: Mann-Whitney Statistical test applied. ns= non-significant; * p≤0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001.

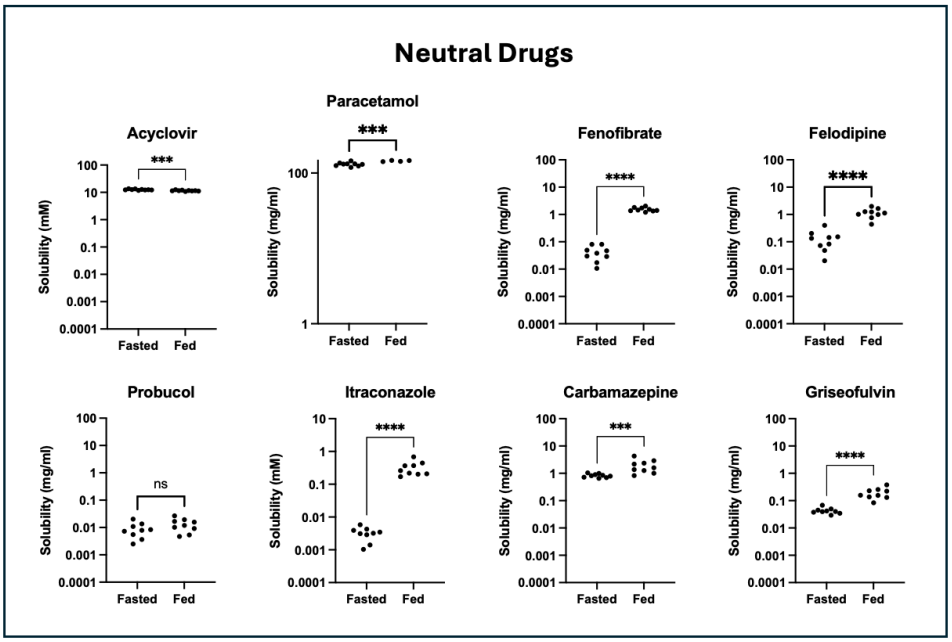


Figure 40. **Solubility comparison fasted vs fed state for neutral drugs**

Note: Mann-Whitney Statistical test applied. ns= non-significant; * p≤0.05; ** p ≤ 0.01; *** p ≤ 0.001; **** p ≤ 0.0001.

Dipyridamole, atazanavir, posaconazole, griseofulvin, carbamazepine, and itraconazole are examples of drugs exhibiting significantly increased solubility from the fasted to fed state, which are also well known for presenting positive food effects. This suggests that the changes in media composition between the two states might lead to increased solubilisation, ultimately impacting overall drug absorption and bioavailability. However, it has also been observed that for some neutral drugs, media composition has little impact on drug solubility variability [129, 246], reflected in very narrow solubility ranges. Acyclovir and Paracetamol (Figure 40) exemplify this behaviour, which was consistent in both fasted and fed states and is likely related to their planar molecular structure. A visual comparison of the neutral graphs (Figure 40) also highlights that the solubility variability of some drugs can change significantly between the fasted and fed states. Fenofibrate and felodipine appear to exhibit less variability in the fed state, with narrower solubility ranges. This supports a previous study that suggested a possible solubility smoothing behaviour due to fed state characteristics [244].

The overall increase in solubility noted for basic and neutral drugs and the variability changes observed in some neutral drugs may indicate that these classes are more susceptible to possible food effects than acidic drugs. The changes in solubility behaviour observed in these multipoint Fa/FeSSIF systems are challenging to detect with single fasted/fed solubility measurements and represent a significant advantage of the application of these systems. Although changes in solubility behaviour cannot fully predict changes in the bioavailability of drugs, they can serve as an indication of food effects and be used for the initial screening of candidate drugs. Associating solubility data with other factors known to affect drug absorption and bioavailability may result in an improved predictive solution for drug food effects.

7.3.2. Oral Dose/Solubility Limited Absorbable Dose: Fasted vs Fed Population Distribution and the Impact of Media Composition Changes

In an effort to correlate the solubility values with other factors that could affect drug absorption and bioavailability, the SLAD was computed for all drugs in both fasted and fed states. SLAD is a biopharmaceutical calculation proposed by the DCS, relating solubility to permeability and other intestinal factors influencing drug absorption. It is determined using the equation:

$$SLAD = s_{INT} \times V \times A$$

Here, S_{INT} represents the intestinal solubility (mg/ml), V is the volume of fasted/fed intestinal fluid, and An is the absorption number:

$$An = \frac{P_{eff} \times T_{si}}{R}$$

Where P_{eff} stands for the effective permeability of the intestine to the drug, T_{si} is the small intestinal transit time (3.32 h), and R is the intestinal radius (1.25 cm). The total volume of intestinal fluid has been calculated as 500 ml for the fasted state and 1150 ml for the fed state, based on the fasted volume plus the volume administered during the fed phase of the intestinal fluid sampling study [89] (400 ml Ensure Plus + 250 ml water). This increase in volume in the fed state compared to the fasted state means that the calculated fed SLAD will be 2.3 times higher than the fasted value, even if the measured solubility of the drug remains unchanged. This calculation was applied to all 9 points of both fasted and fed systems, and the Peff values were obtained from the literature.

The administered dose for each drug was divided by the SLAD values (Dose/SLAD) and applied to generate a plot illustrating the population distribution of intestinal solubility, as depicted in Figures 41-43. A threshold was drawn at 1 where the oral dose and SLAD were equal. The positioning of the fasted and fed curves in relation to the

Dose/SLAD threshold helps understand the percentage of the population that might encounter issues with drug absorption. Dose/SLAD < 1 indicates that intestinal solubility, permeability is adequate for complete absorption in the duration of intestinal transit time (3.32 h), while Dose/SLAD > 1 suggests that intestinal solubility is insufficient for complete absorption in this period. Additionally, the direct comparison of the fasted and fed curves allows for establishing whether the changes in solubility between states have an impact on drug absorption. The impact of solubility changes from the fasted state to the fed state was determined by calculating the total area between the two curves (shadowed area in Figure 41 to Figure 43) in an attempt to measure the shift between the two curves. In order to calculate this, the area between each pair of adjacent points from the fasted and fed curves was represented as a quadrilateral defined by two points from the fasted curve and two points from the fed curve. The area of each quadrilateral was calculated using Gauss's area formula [247]. The quadrilaterals were plotted along with the fasted and fed curves to show the regions where the areas were computed. The total area was calculated as the sum of all quadrilaterals.

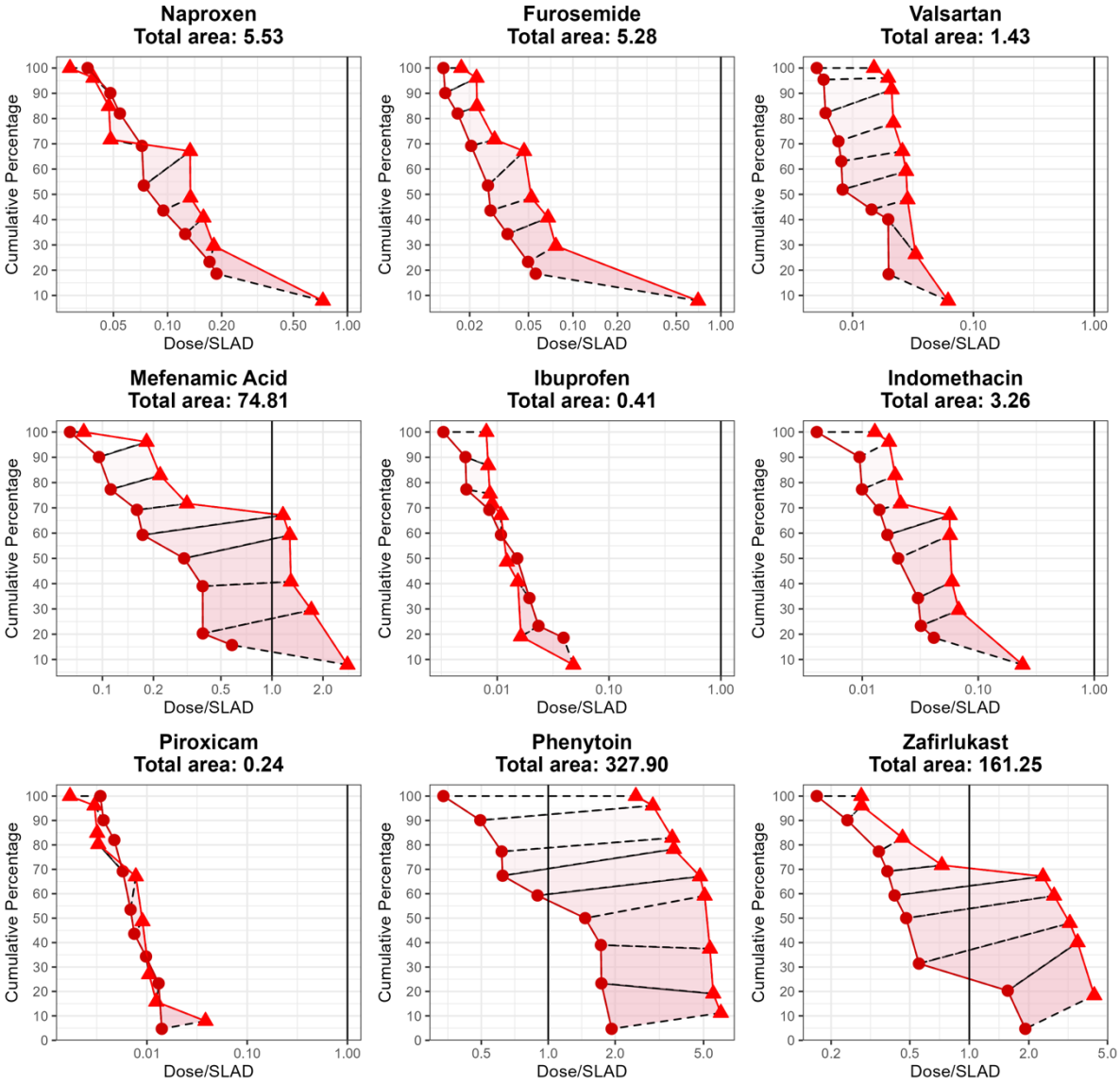


Figure 41. **Cumulative percentage distribution of Dose/SLAD in the fasted and fed state for the acidic drugs.**

Note: Δ Fasted distribution; • fed distribution; Shadowed area represents the total area between the two curves. Quadrilaterals were defined between two consecutive fasted points and two consecutive fed points. Area of each quadrilateral calculated using Gauss's area formula[247]. Total area obtained from the sum of all quadrilaterals' areas.

When examining the examples of acidic drugs, only the curves of mefenamic acid, phenytoin, and zafirlukast intersect the Dose/SLAD limit (Figure 41). For mefenamic acid, phenytoin, and zafirlukast, 67%, 100%, and 70% (respectively) of their fasted population lies above the threshold, suggesting that these drugs may face absorption issues in the fasted state due to a solubility deficit. However, in the case of phenytoin, 50% of the fed

curve remains completely below the threshold, while for zafirlukast, it is 80%, and for mefenamic acid, 100% of the fed population falls below the limit. The shift and separation of the fasted and fed curves for these drugs indicate a solubility difference induced by the fed state, which appears to enhance drug absorption for these drugs. For mefenamic acid and zafirlukast, the solubility comparison alone (see previous section) did not yield statistically significant results, suggesting that the observed change from fasted to fed state might be attributed to the influence of other intestinal factors considered in the SLAD calculation.

All other acidic drugs seem to present both curves below the Dose/SLAD threshold and thus exhibiting an excess of intestinal solubility thereby facilitating complete absorption of the drugs in the duration of intestinal transit time.

Overall, for the acidic drugs, the shift induced by the fed state was either small (mefenamic acid, phenytoin, and zafirlukast) or non-existent, with fasted and fed curves closely aligned or intersecting. This behaviour supports the discussion in the solubility comparison (see previous section). The acids are less susceptible to the changes in media between both states, since the primary factor influencing their solubility is pH, and the observed pH change between states does not seem to be significant enough to have a substantial impact.

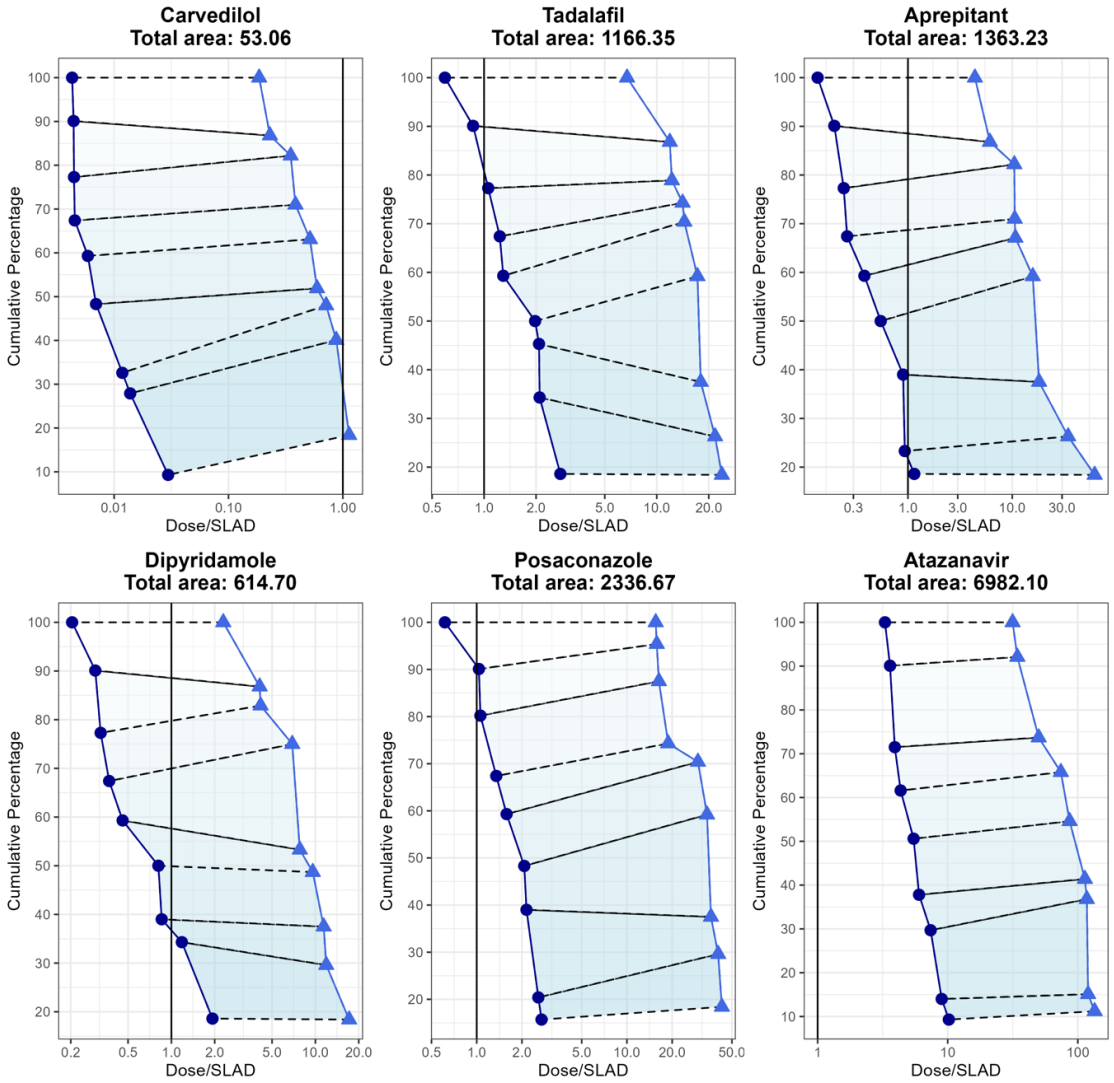


Figure 42. **Cumulative percentage distribution of Dose/SLAD in the fasted and fed state for the basic drugs**

Note: Δ Fasted distribution; • fed distribution; Shadowed area represents the total area between the two curves. Quadrilaterals were defined between two consecutive fasted points and two consecutive fed points. Area of each quadrilateral calculated using Gauss's area formula[247]. Total area obtained from the sum of all quadrilaterals' areas.

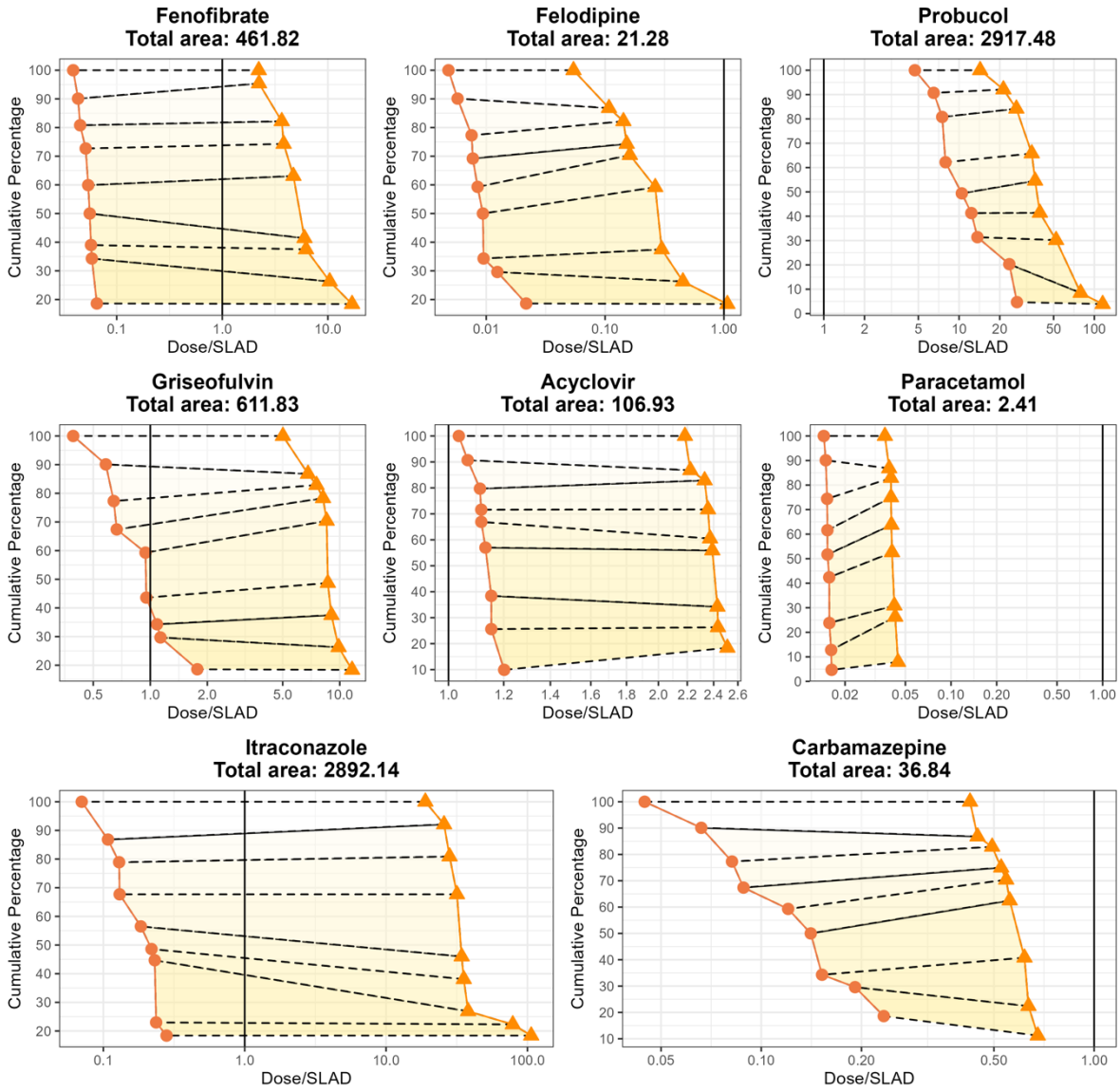


Figure 43. **Cumulative percentage distribution of Dose/SLAD in the fasted and fed state for the neutral drugs**

Note: Δ Fasted distribution; • fed distribution; Shadowed area represents the total area between the two curves. Quadrilaterals were defined between two consecutive fasted points and two consecutive fed points. Area of each quadrilateral calculated using Gauss's area formula[247]. Total area obtained from the sum of all quadrilaterals' areas.

Analysing the fasted and fed curves for the basic drugs reveals a significant shift from the fasted to the fed state, with curves separated by at least a tenfold difference. For dipyridamole and aprepitant, this shift may reduce absorption issues that could arise from the solubility deficit in the fasted state, as evidenced by their fed curves lying below the Dose/SLAD limit. However, for atazanavir, tadalafil, and posaconazole, despite the considerable shift between states, the improvement in the fasted solubility deficit is

limited, with only 20% of tadalafil's fed population and a 10% of posaconazole's placing bellow the Dose/SLAD= 1 threshold. In the case of atazanavir, the changes in the fed state are insufficient, and both states exhibit a solubility deficit that could hinder absorption. The opposite scenario is observed for carvedilol, where although a significant shift is evident between the two states, both curves remain below the threshold, indicating that its solubility is adequate for absorption in both states.

Similar behaviour is observed for certain neutral drugs, where a notable shift in solubility is evident. An approximately 100-fold shift can be observed for fenofibrate, and an even more substantial shift, approximately 1000-fold, is evident for itraconazole. These significant shifts indicate a change in behaviour between states, which aligns with the significantly increased solubility observed in the previous section. A tenfold shift was also observed for griseofulvin, underscoring the significant increase in fed solubility discussed earlier. For these drugs, the shift in solubility implies that the solubility deficit noted in the fasted state appears to be resolved under fed state conditions. What is noteworthy is that these drugs are well known examples of drugs exhibiting in vivo food effects, suggesting that the shift observed in these systems may serve as an *in vitro* representation of the in vivo food effect.

While the solubility difference between the two states was significant for most of the neutral drugs (as discussed in the previous section), not all exhibit substantial changes. The curves for paracetamol, acyclovir, and carbamazepine are closely aligned and show a significantly smaller shift compared to the rest of the neutral drugs. This aligns with the discussion in the previous section and in previous studies [129, 248], where it was observed that acyclovir and paracetamol have narrow solubility ranges, suggesting that their solubility behaviour is not greatly influenced by variations in media components. Similar to carvedilol (as mentioned above), the curves for felodipine, carbamazepine, and paracetamol are positioned below the Dose/SLAD threshold, indicating an excess of solubility and hence no absorption issues. Conversely, for probucol and acyclovir, both curves surpass the threshold, highlighting the solubility deficit likely stemming from their very low solubility.

When comparing basic and neutral drugs with acidic drugs, it becomes evident that they are significantly more affected by changes in the media, resulting in considerably larger shifts between the fasted and fed curves. Since the solubility of these

drugs relies on the media's amphiphilic components, a solubility based food effect is almost inevitable, as evidenced by Figure 41 to Figure 43. It is worth noting that although both neutral and basic drugs exhibit significant differences between states, basic drugs tend to show a more consistent increase, with almost all examples presenting around a tenfold difference between the fasted and fed states. In contrast, the shift varies among neutral drugs. Considering that basic drugs are still influenced by the pH of the media but to a lesser extent than acids, the insignificant pH difference between the two states may also play a role in diluting the impact of media composition variation for basic drugs, resulting in smaller increases compared to neutral drugs.

This fasted and fed Dose/SLAD analysis could serve as a tool in drug development, establishing a baseline performance threshold based on solubility to ensure complete absorption at the administered dosage in both states. It aids in evaluating if fasted state solubility issues could be resolved in the fed state and whether statistically significant solubility changes between states are relevant for drug absorption. The observed shift between curves, particularly noteworthy in cases like griseofulvin, fenofibrate, and itraconazole, may be linked to food effects. A solubility shift is more likely to lead to a food effect if the drug encounters absorption issues, or in this system, if one or both of its curves surpass the Dose/SLAD threshold. Conversely, if the drug exhibits sufficient solubility, with both curves falling below the threshold, then food effects are less likely to be a concern, and it is improbable that the drug will experience absorption issues related to food.

7.3.3 Correlating Curve Shifts With In Vivo Food Effects

In an attempt to correlate the shift between the curves with in vivo food effect data, the total area (calculated as described in the previous section) was plotted with the in vivo ratio of bioavailability (fed/fasted) (Figure 44). The total area was chosen as a measure of the magnitude of the shift between the fasted and fed curve. Since the points from the fasted and fed curves are not paired, it is difficult to calculate the shift of each of the nine points. Therefore, the approach was to calculate the area of the

graphical space between these two curves and use it as the magnitude of the shift (Figure 41 to Figure 43).

As previously stated, a food effect was considered by the FDA if the 90% confidence intervals for the ratio of population geometric means for C_{max} and AUC between fed and fasted states fall outside the 80–125% bioequivalence limits established for the fasted state. Thus in Figure 44, the bioavailability ratio was calculated as the ratio of fed AUC and fasted AUC and the areas bellow 0.8 and above 1.25 were marked in red to highlight the FDA food effect threshold.

Visual inspection of the figure appears to show a positive correlation between the area between the curves and the bioavailability ratio. Drugs with larger areas between the fasted and fed solubility curves (mostly neutral and basic drugs) are predominantly situated outside the FDA food effect threshold. Conversely the majority of acidic drugs with smaller solubility shifts are located inside the food effect threshold.

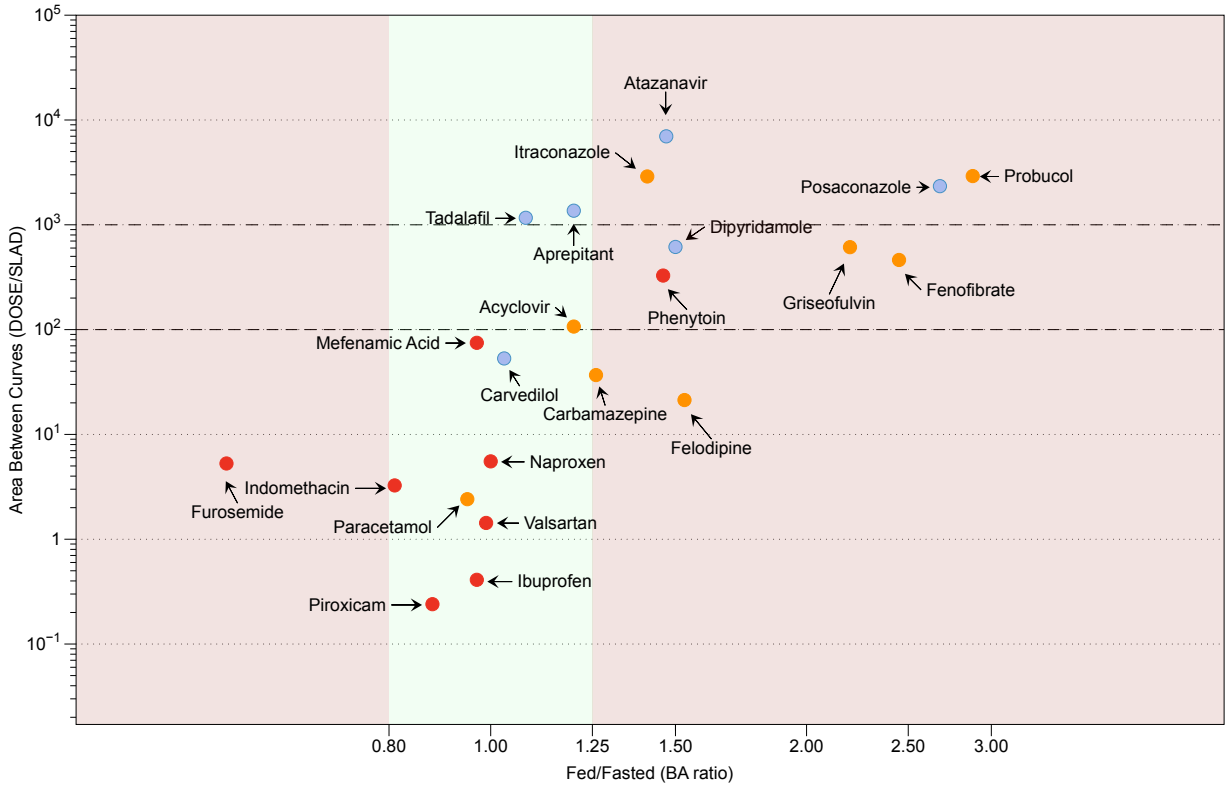


Figure 44. ***In vivo* bioavailability (BA) ratio (food effect) vs total area between the curves**.
Note: BA ratio: $\frac{AUC_{FED}}{AUC_{FASTED}}$; No food effect: BA ratio between 0.8 and 1.25; Food effect: BA ratio below 0.8 and above 1.25.

Spearman correlation coefficients were calculated for each drug class (acidic, neutral and basic). Spearman correlation was chosen over regression due to the nature (in vivo vs *in vitro*) of the datasets. The bioavailability data that resulted in the food effect ratio encompass multiple in vivo biological variables that may not align directly with *in vitro* solubility shifts. Spearman's rank correlation, which identifies relationships without assuming linearity, was better suited to capture the association between solubility changes and food effects across different drug classes. Neutral drugs exhibited the strongest correlation (r = 0.810), suggesting that solubility shifts in biorelevant media could be predictive of their food effects. Basic drugs showed moderate correlation (r = 0.543), while acidic drugs showed the weakest correlation (r = 0.443). Overall correlation (r=0.775) suggests a notable link between increasing solubility shifts and in vivo food effects. This aligns with the discussion in the previous section.

7.3.3. *In vitro* prediction of food effects

Relying solely on solubility as a predictor of food effects risks underestimating or overestimating these impacts. As illustrated in Figure 45, solubility is just one of many factors influenced by food. Drug absorption, permeability, dissolution, transporter activity, and metabolism are also significantly affected.

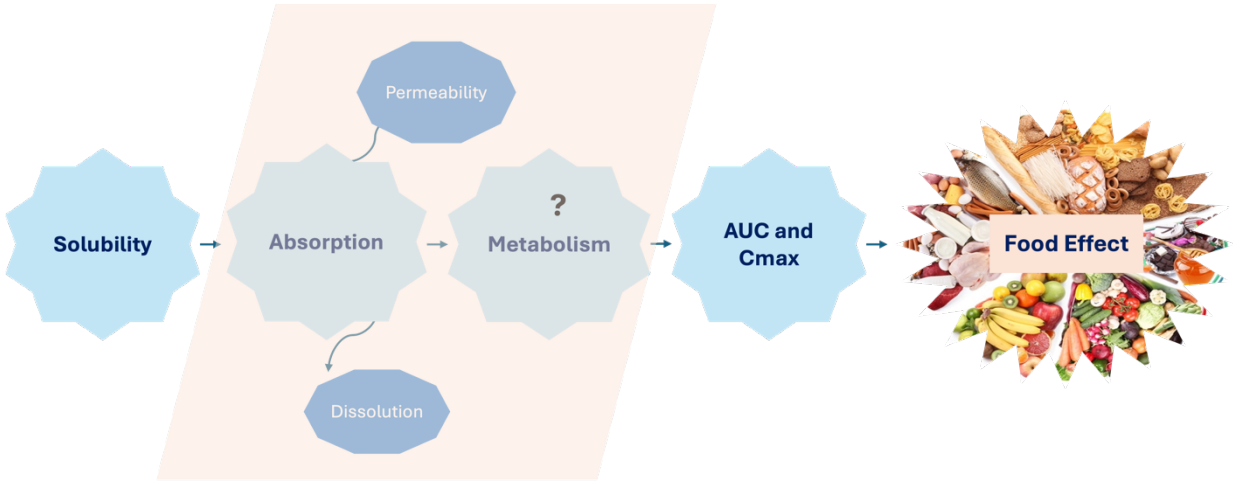


Figure 45. **Factors that are influenced by the presence of food.**

By applying solubility data from the enhanced fasted and fed biorelevant systems and statistically comparing it with observed food effects (Table 18), this study achieved

a prediction accuracy of 69.5%. However, as discussed earlier, incorporating additional intestinal factors, such as those highlighted by the SLAD approach, could improve prediction accuracy. The Dose/SLAD approach described in the previous sections, combined administered dose with solubility limits and population distributions and enabled the identification of shifts between fasted and fed states, that can be calculated as the total area and used as the magnitude of the change between the two states. Drugs known to exhibit food effects, such as griseofulvin, fenofibrate, and itraconazole, displayed substantial shifts, with fasted curves above the Dose/SLAD threshold. Conversely, drugs without known food effects, such as ibuprofen and paracetamol, exhibited closely aligned curves with no significant shifts, and both curves below the Dose/SLAD threshold. Therefore, these systems could potentially be useful as an *in vitro* tool for predicting solubility-related food effects.

To better predict food effects, developing a predictive model using AI or machine learning tools could allow for more comprehensive analysis of the available data. However, the current dataset, limited to 23 drugs, poses challenges for building robust AI models. As an alternative, creating a decision tree could be a practical and efficient solution. This approach uses the existing data to systematically categorise drug behaviours, offering a structured predictive tool for food effects despite the small dataset.

To enhance the accuracy of the prediction model, a stepwise decision tree approach was applied. The following outlines the criteria created to construct the decision tree (Table 18, Figure 46):

1. **Solubility data**: Statistical comparison of solubility values in the fasted and fed states, using the Mann-Whitney test. Significant effect considered if $p<0.05$. Solubility was treated as a gatekeeper parameter. Thus, instead of rejecting drugs outright when solubility does not reveal a statistically significant difference, a re-evaluation step was introduced for cases where solubility alone may not determine the outcome.
2. **Dose/SLAD Categories**: There are three types of behaviour that the fasted and fed curves can present in the Dose/SLAD approach. Curve below the Dose/SLAD threshold (<1), above the Dose/SLAD threshold (>1) and crossing the Dose/SLAD threshold. In order to fit the data into the model, crossing curves were consider

>1 or <1 depending on population distribution. If a curve presents the majority of its population (>50%) above the threshold it should be considered as >1 and vice versa. If the fasted or fed curve is located > 1, it suggests inadequate solubility for complete absorption thus a food effect was considered. If the fasted and fed state curves are both located < 1 it suggests sufficient solubility for absorption thus, no food effect was considered.

3. **Thresholds for Area Between Curves**: The area between the curves was categorised into three ranges. These thresholds were based on the analysis of Figure 44 where 73% of drugs without in vivo food effects presented total areas lower than 100. These categories were used to refine the predictions of the decision tree, making the model sensitive to the degree of change in drug absorption caused by food intake.
    - **Low Area (≤ 100)**: Minimal shift in absorption, weak or no food effect.
    - **Moderate Area (100–1000)**: Potentially significant food effect.
    - **High Area (> 1000)**: Large shift in absorption, strong food effect likely.
4. **Metabolism**: In an attempt to include non-solubility-related factors as steps in the decision tree, metabolism was taken into account in this model. Clint was used as a measure of metabolism. Low metabolism was considered for Clint values 10-100, Intermediate metabolism if values between 100-1000 and High if 1000-> 10000 [249]. The adopted decision tree criteria for metabolism were:
    - **High metabolism** drugs are more likely to be influenced by food (based on the example of atazanavir and ritonavir both with high metabolism and well reported food effect (See Table 18)).
    - **Intermediate** drugs likely to be influenced by food if associated with a statistical diference in solubility and moderate to high area between the curves.
    - **Low metabolism** drugs are less likely to have a food effect.

After applying the decision tree logic to each drug (Figure 46), the predicted food effect was compared against the actual in vivo food effect as reported in the literature

(Table 18). The accuracy of the predictive model was assessed by determining how many drugs had their predicted food effects matching the actual outcomes.

The comparison of the decision tree predictions with the literature yielded an agreement of 82.6% (83%). When examining the solubility agreement by drug category, it was found to be 77.8% for acidic drugs, 87.5% for neutrals, and 83.3% for basic drugs. This approach was generally successful in predicting potential food effects in drugs known to exhibit them, as well as in accurately predicting the absence of food effects in drugs that do not demonstrate them in vivo. However, some drugs may have a food effect driven by mechanisms not captured in the current decision tree. Tadalafil presents a large shift between the curves (1166.35) which suggests a strong food effect, but actual in vivo studies suggest no food effects were found which could indicate a unique mechanism at play not covered by the prediction model. Furosemide presents a very small area (5.28) and low metabolism which suggested minimal food effect, yet it has a reported in vivo food effect.

Overall, the combination of solubility data, Dose/SLAD category, total area between the curves and metabolism, improves upon solubility predictions alone. The high prediction rate observed is promising in terms of equivalence with in vivo behaviours, especially considering that it is still mainly solubility based with the inclusion of some intestinal factors (SLAD). This decision tree model could serve as a useful starting point for drug formulation. However, in order to validate this model, a larger dataset should be studied, and additional criteria must be included to help fill the gap between solubility and food effects (Figure 45) and refine the thresholds established. This approach could be applied to a larger dataset which would enable integration into PBPK models, allowing for the inclusion of additional pharmacokinetic parameters such as intestinal transit, absorption rates, and systemic metabolism. These computational approaches could provide a more comprehensive framework for predicting food effects and improving *in vitro*-in vivo correlations, ultimately enhancing drug development strategies.

Table 18. **Selected criteria to construct a decision tree and prediction results**

	Solubility (Significant Difference)	**Dose/SLAD** (Category)		**Dose/SLAD** (Area between curves)	**Metabolism** (Clint)	**Food Effect** (in vivo)	**Food Effect Prediction** (Decision tree)	
		Fed	**Fasted**					
	Neutral Drugs							
Acyclovir	Yes	>1	>1	106.93	Low	Yes [250]	Yes	✅
Paracetamol	Yes	<1	<1	2.41	Low	No [251, 252]	No	✅
Fenofibrate	Yes	<1	>1	461.82	-	Yes [253]	Yes	✅
Felodipine	Yes	<1	<1	21.28	High	Yes [254, 255]	Yes	✅
Probucol	No	>1	>1	2917.48	-	Yes [256]	Yes	✅
Itraconazole	Yes	<1	>1	2892.14	-	Yes [257]	Yes	✅
Carbamazepine	Yes	<1	<1	36.94	Low	Yes [258]	No	❌
Griseofulvin	Yes	<1	>1	611.3	-	Yes [259, 260]	Yes	✅
	Basic Drugs							
Aprepitant	Yes	<1	>1	1363.23	Low	Yes [261]	Yes	✅
Carvedilol	Yes	<1	<1	53.06	Intermediate	No [262]	No	✅
Tadalafil	Yes	>1	>1	1166.35	-	No [263]	Yes	❌
Dipyridamole	Yes	<1	>1	614.70	Intermediate	Yes [190, 264]	Yes	✅
Atazanavir	Yes	>1	>1	6982.10	High	Yes [265]	Yes	✅
Posaconazole	Yes	>1	>1	2336.67	Intermediate	Yes [266, 267]	Yes	✅
	Acidic Drugs							
Valsartan	No	<1	<1	1.43	Low	No [268]	No	✅
Zafirlukast	No	<1	>1	161.25	Low	Yes [269, 270]	Yes	✅
Ibuprofen	No	<1	<1	0.41	Low	No [251, 271, 272]	No	✅

Furosemide	No	<1	<1	5.28	Low	Yes [188, 273]	No	❌
Mefenamic Acid	No	<1	>1	74.81	Low	No [274]	Yes	❌
Piroxicam	Yes	<1	<1	0.24	Low	No [275]	No	✅
Phenytoin	Yes	>1	>1	327.90	Low	Yes [276, 277]	Yes	✅
Indomethacin	No	<1	<1	3.26	Low	No [251, 278]	No	✅
Naproxen	No	<1	<1	5.53	Low	No [251, 279]	No	✅
Solubility: Mann-Whittney statistical test Area between the curves: as described in Section 7.3.2 Metabolism: CL int (ml/min/kg): Low:10-100/ Intermediate: 100-1000/High: 1000-> 10000 [249]							**Prediction Success** 82.6%	19/23 drugs

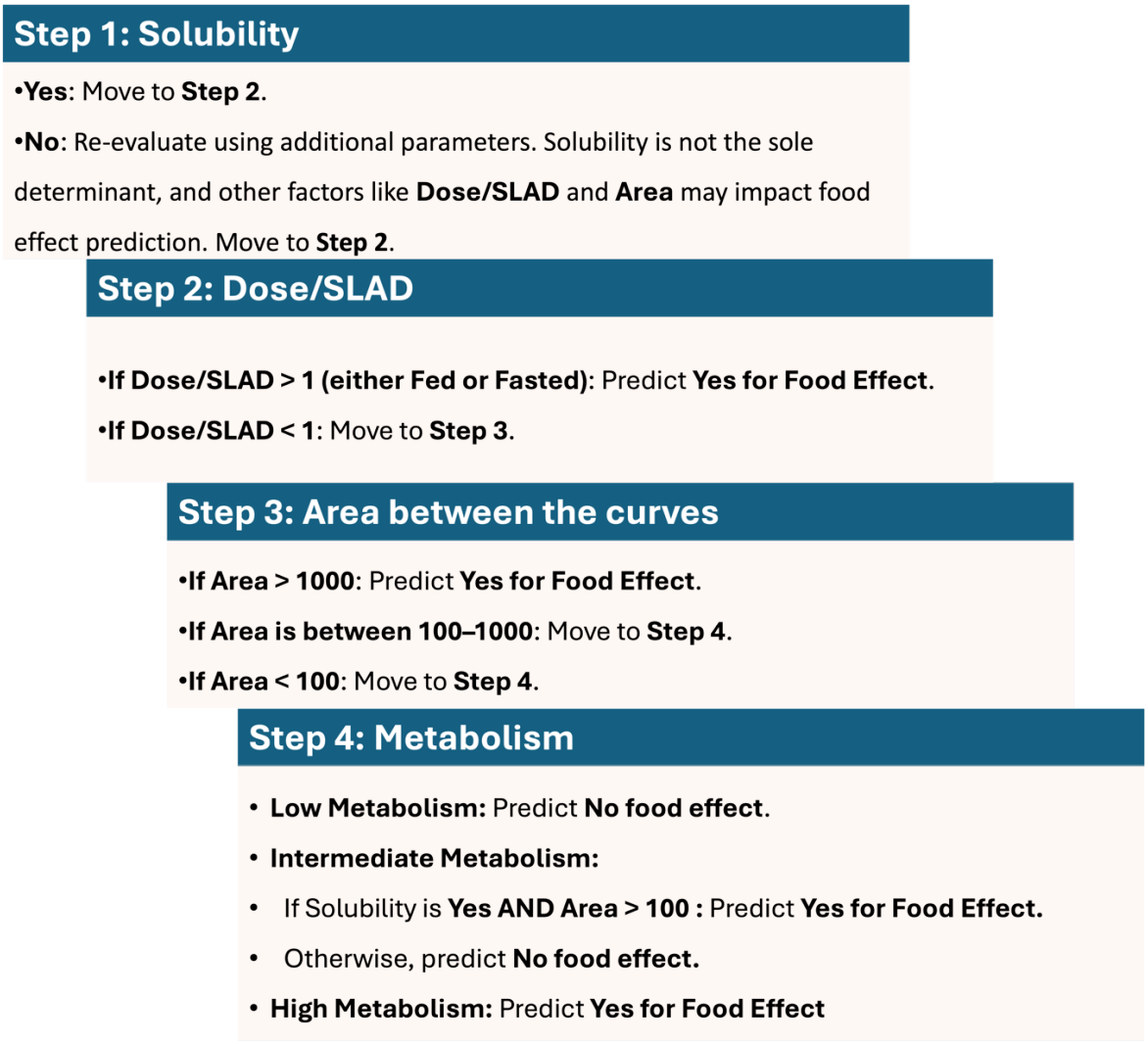


Figure 46. **Decision tree logic and stepwise structure.**

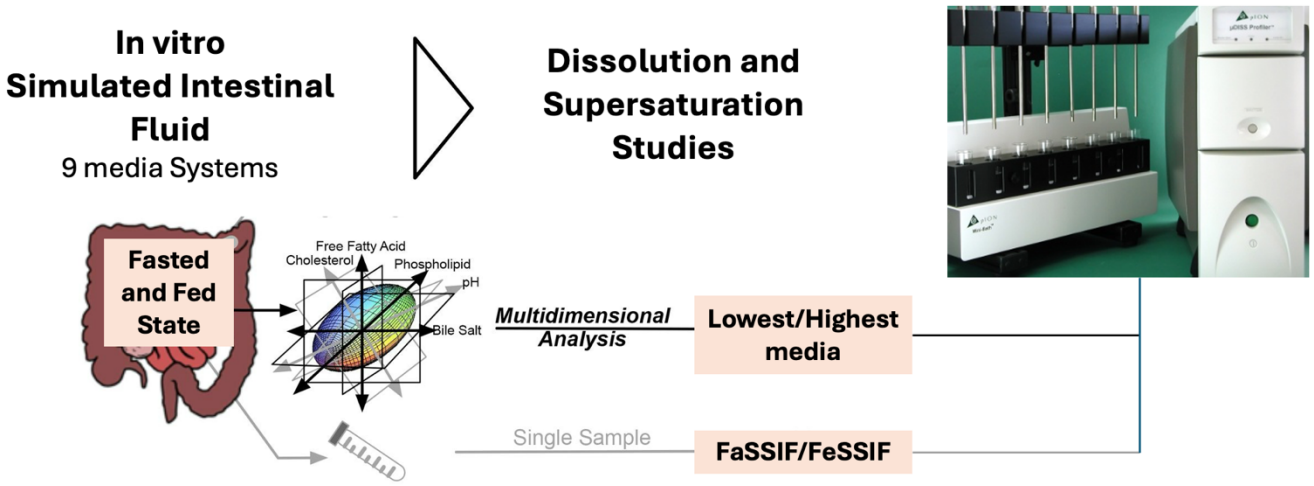
7.4. Conclusion

This study highlighted the solubility differences of acidic, basic, and neutral drugs between fasted and fed biorelevant media. Acidic drugs generally displayed minimal solubility differences between the states, with pH being a key factor, while basic and neutral drugs exhibited significant solubility increases in the fed state, driven by higher TAC. The incorporation of solubility data with intestinal factors, such as permeability and transit time, through the SLAD calculation provides a valuable framework for predicting drug absorption and bioavailability. The Dose/SLAD analysis revealed that acidic drugs generally exhibit minimal solubility differences between fasted and fed states, while basic and neutral drugs showed substantial shifts. These shifts align with known food

effects for certain drugs, indicating the potential of these multipoint systems to predict absorption-related food effects. A positive correlation between the area separating fasted and fed solubility curves and in vivo food effects was observed, with neutral drugs showing the strongest correlation (r = 0.810), followed by basic drugs (r = 0.543), and acidic drugs (r = 0.443). This suggests that solubility shifts in biorelevant media are particularly predictive of food effects for neutral drugs. The overall correlation (r = 0.775) highlights the utility of solubility shifts as an *in vitro* indicator of potential in vivo food effects. A decision tree was developed to predict drug food effects, addressing the limitations of relying solely on solubility. The decision tree systematically incorporated solubility data, Dose/SLAD thresholds, the area between fasted and fed curves, and metabolism as key predictors. The model achieved 83% accuracy in predicting food effects, outperforming solubility predictions. Expanding the dataset and including additional pharmacokinetic factors could further improve prediction reliability and refine the model's thresholds.

Chapter 8

Applying Biorelevant Simulated Intestinal Media to the Study of Dissolution and Supersaturation of Drugs



This chapter contains data collected during a 6 week placement at GSK Stevenage under the supervision of Dr. Wayne Matthews.

8.1. Introduction

8.1.1 Dissolution Testing: Compendial or Biorelevant Conditions?

As discussed in the previous chapters, solubility and dissolution of drugs are critical factors in determining their bioavailability and therapeutic success. For drugs that exhibit poor solubility such as BCS Class II and DCS Class IIa, the rate-determining step to absorption is often considered to be dissolution in the small intestine [280-282].

Traditionally, *in vitro* dissolution testing has been used to assess the performance of oral drug formulations. However, conventional compendial dissolution experiments have limitations since they fail to accurately reflect physiological conditions. This is mainly because traditional dissolution tests are designed to assess the quality of the dosage form and its ability to release the drug, rather than fully account for the physiological conditions of the gastrointestinal tract (such as pH variations, bile salt concentrations, fluid dynamics, GI transit time, and digestive enzymes), which significantly influence drug dissolution after oral administration [283]. The use of biorelevant media for dissolution testing was first proposed by Dressman *et al*. in 1998 [112] and it accounted for the variations in pH, ionic strength, and concentrations of buffers and bile components that occur throughout the gastrointestinal tract. However, there has been ongoing debate about the most appropriate media and methods to use in order to achieve biorelevant and reproduceable dissolution data that accurately reflect in vivo conditions, however no consensus has yet been reached on a universal approach [112, 284-286].

The importance of using a more biorelevant media for dissolution testing is reiterated by the role of colloidal structures such as micelles and vesicles formed by bile components in the solubilisation and thus dissolution rate of drugs [287]. Increasing bio relevancy of the testing media in solubility studies improved *in vivo- in vitro* correlations (IVIVC)[128], revealed drug-specific solubility behaviours [129, 248], underlined solubility changes from the fasted to fed state[245] and highlighted solubility spaces not covered by the traditional single measurement FaSSIF/FeSSIF approaches [161, 243-

245]. The association between solubility and dissolution rate explained by the Noyes-Whitney equation [104] (See Chapter 1) states that increasing solubility and surface area of a drug will lead to a higher dissolution rate. Therefore, dissolution media with different biorelevant components either because of the multiple recipes available for SIF media or in an attempt to make changes representative of the fasted and fed state, will most likely result in variations in the dissolution profile of drugs similarly to what was found for solubility (See Chapter 2 to Chapter 6).

8.1.2 Measuring Dissolution Rate in Various Complex Media

In order to study dissolution, multiple methods have been developed to assess dissolution rate under conditions that differ from the compendial dissolution assays. The IDR (μg/min/cm²) provides a more standardised way to measure dissolution, as it normalises the dissolution rate based on the surface area of the API[287]. As an inherent property, IDR is useful in various contexts, such as selection of a certain solid state form (e.g., salt, co-crystal, or polymorph)[288], enabling formulation strategy (e.g., solid dispersion or cyclodextrin inclusion) [289, 290] or optimise particle size to ensure complete dissolution of a certain drug dose during intestinal transit [291]. Typically, IDR is determined by using a rotating disc of compacted powder with a fixed surface area, which is placed in contact with the dissolution media [292]. In early drug development, formulation scientists often have access to only a limited amount of API. As a result, significant research has focused on creating miniaturised experimental assays that enable high-throughput measurement of dissolution rate using minimal quantities of material [134]. The primary techniques and equipment used include:

- miniaturised dissolution vessels, flow-through cells, or microtiter plate-based methods where API concentration changes are typically measured offline [293].
- imaging-based techniques [294].
- small scale dissolution instruments that utilise UV fibre optic probes for *in situ* API concentration measurement [295, 296].

Considering the available equipment at the University of Strathclyde and the characteristics of the media under study, *in situ* UV fibre optic probes were selected to measure drug concentration over time during dissolution. The media used for these studies were fasted and fed biorelevant SIF media, previously applied in the solubility studies described in Chapter 2, Chapter 3, Chapter 4, and Chapter 5. Figure 47 shows the appearance of the nine different media for the fasted and fed states, and their appearance 1 and 24 hours after the addition of the API, while maintained at 37°C.

In order to proceed with dissolution testing using these SIF media, it was decided that, given their appearance and turbidity changes over time, and considering that the planned assay duration would match the intestinal transit time (3 hours), it would be sensible to start dissolution testing with *in situ* measurements. This approach would account for any media variations, as spectra could be collected very frequently (every second). One commonly used equipment that was considered appropriate to start this study was the SiriusT3 (Pion Inc., Billerica, MA, USA), a fully automated system with auto-titration and sample autoloader modules. This equipment typically allows dissolution analysis with API sample amounts ranging from 5–10 mg and is equipped with a UV probe for *in situ* measurements. However, after a few trials, it became evident that the Sirius T3 probe could not detect the drug due to the media's turbidity, even in the fasted state. Since drug detection was not possible, no results were collected and thus were not included.

Another piece of equipment available at the university was the inForm (Pion Inc., Billerica, MA, USA), a fully automated system similar to the Sirius T3 but capable of analysing up to 100 mg of API with dissolution media volumes ranging from 3 to 80 mL. It was important to maintain a lower volume required for the dissolution testing in order to reduce the waste of the components used in the production of these media. The great advantage of the inForm equipment was that its more advanced UV probe, allowed for adjustable path lengths from 5 mm to 10 mm, enabling optimisation for each drug as needed. Although the inForm system was able to perform dissolution measurements using FaSSIF, it still encountered difficulties with the biorelevant SIF media and with FeSSIF(data not included). The equipment that seemed more appropriate for dissolution measurements using these complex media but that was not available at the university was the µDiss Profiler (Pion Inc., Billerica, MA, USA). Its significant advantage was the

inclusion of multiple, more advanced probes, with adjustable path lengths ranging from 1 mm to 20 mm, offering greater flexibility to customise the assay depending on the drug and media being studied.

The dissolution data presented in this chapter was obtained at GSK Stevenage using the µDiss Profiler (Pion Inc., Billerica, MA, USA). For this study, the biorelevant media used in previous solubility studies (see Chapter 2, Chapter 3, Chapter 4, and Chapter 5) were selected. However, due to time constraints, not all nine media for each state were tested. Instead, the focus was on the media that resulted in the lowest (worst case scenario) and highest (best-case scenario) solubility values. In the earlier solubility studies (see Chapter 2 and Chapter 3), these media corresponded to the lowest and highest TAC, which represents the combined concentrations of all media components in both fasted and fed states. Therefore, Media 1 and Media 2 (Table 6 and Table 7) for both fasted and fed states were chosen for the dissolution testing. A comparison with the commonly used Fa/FeSSIF was also performed. The aim was to study the impact of media changes on the drug's IDR and to correlate these findings with the previous solubility data. The drugs selected for this study were dipyridamole, griseofulvin, and tadalafil, with their characteristics presented in Table 8. These drugs were previously applied to the solubility studies in both fasted and fed state (See Chapter 6) and selected as examples of drugs that presented few changes in solubility between the fasted and fed state (Tadalafil) and noticeable changes in solubility (Dipyridamole and Griseofulvin).

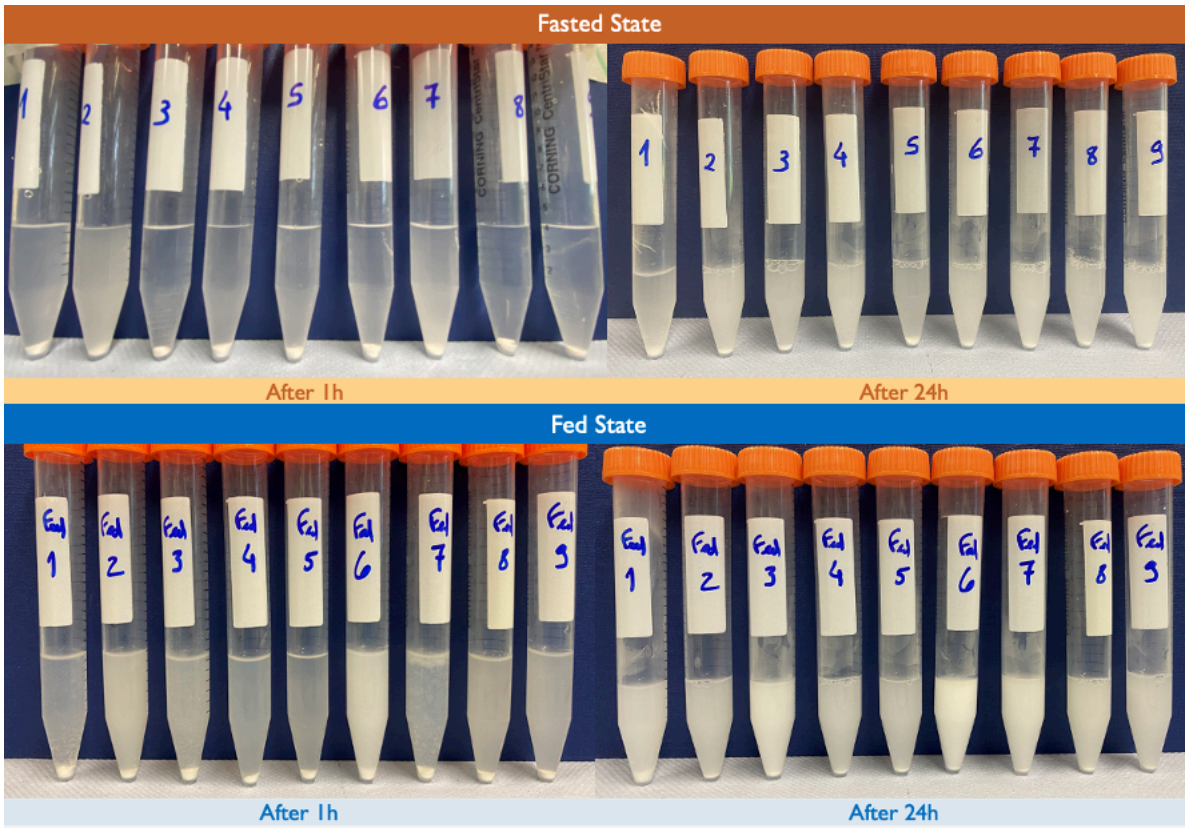
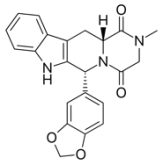
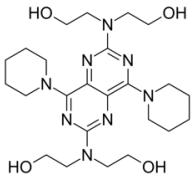
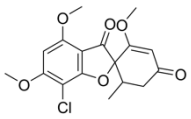


Figure 47. **Biorelevant SIF media appearance in the fasted and fed state**

Note: Comparison of the media changes after 1 and 24 hours of addition of an API. The precipitate visible in all tubes corresponds to the excess of API (fenofibrate) added during previous solubility studies.

Table 19. **Physicochemical properties and molecular structures of the drugs in the dissolution study**

Compound	a/b/n	pKa	Log P	Structure
Tadalafil	b	3.5	1.7	
Dipyridamole	b	6.2	3.77	

Griseofulvin	n	–	2.18	

Note: a/b/n representing acids, neutrals and basic drugs respectively.

8.1.3 Supersaturation and Supersaturating Drug Delivery Systems

As underlined in the previous chapters, poor solubility might jeopardise drug absorption and hinder therapeutic success. In recent years, there has been growing interest in the role of intestinal supersaturation as a key mechanism to enhance the bioavailability of poorly soluble drugs [297, 298]. By maintaining drug concentrations above their solubility threshold in the intestinal lumen, supersaturation presents an effective strategy to address solubility-related challenges, improving the potential for absorption [299, 300]. However, the generation and maintenance of the supersaturated state are essential steps in this process. Without appropriate control, supersaturation can lead to precipitation, which would reduce the amount of drug available for absorption [299].

Supersaturation is a thermodynamically metastable state that drives precipitation and crystallisation [299-301]. The effectiveness of Supersaturating Drug Delivery Systems (SDDS) in improving bioavailability is closely linked to the stability of this supersaturated state and the rate at which precipitation occurs. Therefore, maintaining supersaturation for a sufficient period is critical to ensuring efficient drug absorption [301]. To better understand the behaviour SDDS, it is important to distinguish between supersaturation and thermodynamic solubilisation as strategies to enhance drug absorption [299]. Thermodynamic solubilisation refers to the process of increasing the solubility of a drug in the GI fluid using solubilising agents, which helps the drug dissolve more easily. This process can modify the drug's physicochemical properties, thereby influencing its tendency to permeate the epithelial membrane.

Supersaturation involves creating a solution where the concentration of dissolved drug exceeds its equilibrium solubility. This strategy increases the free drug concentration in the GI lumen, making more drug available for absorption. Unlike solubilisation, supersaturation does not necessarily alter the drug's permeability through the epithelial membrane. Instead, it enhances the concentration gradient, driving more drug molecules to diffuse through the membrane [299].

This distinction is critical for developing formulations that optimise the supersaturation state and extend its duration before precipitation occurs. If the supersaturated state can be maintained long enough, it can provide more effective absorption than solubilisation alone.

The need to manage this metastable state underscores the importance of evaluating supersaturation and precipitation kinetics when developing and optimising SDDS [302]. Given the practical limitations associated with *in vivo* studies, *in vitro* assays have become crucial in predicting the behaviour of supersaturation and precipitation under biorelevant conditions [303]. Despite advances in solubility and dissolution testing, traditional compendial methods often fall short in predicting the in vivo behaviour of supersaturation and precipitation[299]. To address this, more biorelevant dissolution media, which mimic the conditions in the fasted and fed states of the small intestine, have been developed and significantly enhance the accuracy of *in vitro*-in vivo correlations by providing a better simulation of physiological conditions [299, 302]. Moreover, components such as bile salts and phospholipids in these fluids may influence precipitation kinetics.

The use of SIFs in supersaturation studies offers a more accurate prediction of in vivo drug behaviour and helps optimise formulation strategies for enhanced drug absorption [303]. Studies comparing the precipitation behaviour of poorly soluble drugs in human gastrointestinal fluids and simulated fluids highlight the importance of selecting the appropriate media for supersaturation studies [303, 304]. Research by Bevernage *et al.* [301, 303, 305] revealed that simple aqueous buffers at pH 6.5 can significantly overestimate the stability of supersaturation. While FaSSIF reasonably predicts precipitation in the fasted state, FeSSIF may underestimate precipitation in fed conditions, indicating the need for careful media selection in supersaturation studies.

- **Quantitating Supersaturation through *in vitro* methods**

The *in vitro* evaluation of drug supersaturation involves two essential steps: inducing supersaturation in the chosen media and monitoring drug concentrations over time [306]. Various methods for generating supersaturation include techniques such as solvent evaporation, ion addition, temperature changes, and shifts in pH or solvent. However, for testing the supersaturation potential the most commonly employed methods are solvent and pH-shift methods [299] [307].

The pH-shift method provides an alternative approach to measure supersaturation. This technique reduces drug ionisation by altering the pH, thus lowering solubility and inducing supersaturation [299]. The pH-shift method is considered more biorelevant because it mimics the natural pH changes that occur as drugs transition from the acidic environment of the stomach to the neutral pH of the small intestine especially for weakly basic drugs [101]. It can be applied in either a single-compartment system, where a buffering agent increases the pH above the drug's pKa, or in a two-compartment setup, where a drug solution is transferred from an acidic to a neutral environment, simulating intestinal conditions [101, 308, 309]. This approach provides valuable insights into the supersaturation behaviour of drugs under physiological conditions.

Another technique for studying supersaturation is the CheqSol® system, a potentiometric method used to evaluate ionisable drugs [310]. This system operates by titrating a solution of the drug in its ionised form, with continuous monitoring of pH and UV absorbance. After dissolving the drug by adjusting the pH, a titrant—either KOH for bases or HCl for acids—is added to convert the drug into its less soluble neutral form, inducing supersaturation [299]. Precipitation is detected through increased absorbance, and this process allows for the quantification of supersaturation extent and duration[311]. The CheqSol® method differentiates between drugs with high supersaturation potential, referred to as "chasers," and those that rapidly precipitate, known as "non-chasers." It also assesses the impact of excipients on the stability of the supersaturated state, making it useful in formulating ionisable drugs [310, 311].

The most common method, which is also the focus of this study, is the solvent shift method. This technique, also known as the co-solvent quench method, is a popular and simple approach for creating supersaturation at a constant system pH value [298, 312,

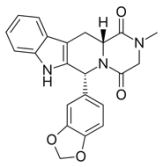
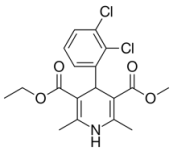
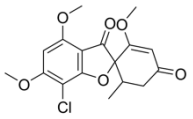
313]. In practice, a poorly water-soluble drug is first dissolved in a water-miscible solvent with a high solubilising capacity, such as DMSO, DMF, or PEG, and is then added to an aqueous medium where supersaturation is induced due to the solubility difference [299, 312]. The DS can be adjusted by controlling the concentration of the drug in the solvent and the amount of solvent transferred to the aqueous medium. Although this method is widely applicable and can be automated for high-throughput screening, its biorelevance is sometimes questioned because it does not replicate the complexity of the gastrointestinal environment[299]. The main difficulty with the solvent shift method lies in selecting an optimal supersaturation level for study, as each compound has a unique tendency to remain supersaturated. Certain compounds can maintain high DS without precipitating immediately, while others tend to precipitate quickly at comparable or even lower DS levels [300]. This hampers literature comparison and results in inconsistent methods to choose DS concentrations.

While significant effort has been dedicated to developing formulations that induce supersaturation, the understanding of how supersaturation behaves in biorelevant environments remains limited. Components in the gastrointestinal tract, such as bile salts, lecithin, and food digestion products, can significantly impact supersaturation stability, but their influence is not yet fully understood [303]. Consequently, there is limited knowledge about how different biorelevant components affect the supersaturation and precipitation of drugs under conditions that mimic the fasted and fed states in the human body [299, 303].

The aim of this study is to investigate drug supersaturation in the same biorelevant SIF media applied in the previous solubility and dissolution studies. The supersaturation method applied was the Standardised Supersaturation and Precipitation Method (SSPM) [135] in an attempt to standardise the choice of DS in each media for the different drugs.

The drugs selected were felodipine, griseofulvin, and tadalafil. Additionally, this study seeks to explore potential food effects on supersaturation by using simulated intestinal media that represent both fasted and fed states, providing a more comprehensive understanding of how biorelevant conditions influence drug supersaturation behaviour.

Table 20**. Physicochemical properties and molecular structures of the drugs in the supersaturation study**

Compound	a/b/n	pKa	Log P	Structure
Tadalafil	b	3.5	1.7	
Felodipine	n	-	3.86	
Griseofulvin	n	–	2.18	

Note: a/b/n representing acids, neutrals and basic drugs respectively.

8.2. Methods

8.2.1. Dissolution

Dissolution experiments were conducted using a µDiss Profiler (Pion Inc., Billerica, MA). The experiments aimed to study the dissolution profiles of griseofulvin, dipyridamole and tadalafil in different biorelevant media by measuring absorbance *in situ* over time.

FaSSIF and FeSSIF were selected as a reference for simulated intestinal media and four biorelevant SIF media representative of the fasted and fed state were used to study the impact of media composition variation on dissolution. Fasted/Fed Media 1 was representative of the media with lower concentration of components referred as total amphiphile content and Fasted/Fed Media 2 was representative of the higher concentration of components (pH*TAC, Table 6 and Table 7).

- **Media Preparation**

As previously described in Section 2.3

- **Preparation of the discs**

As previously described in Section 2.5.1

- **Standard Curves**

As previously described in Section 2.5.2

- **Dissolution Protocol**

As previously described in Section 2.5.3

- **Calculation of Intrinsic Dissolution Rate**

As previously described in Section 2.5.4

8.2.2. Supersaturation and Precipitation

Precipitation experiments were conducted using a µDiss Profiler (Pion Inc., Billerica, MA). The experiments aimed to study the precipitation of felodipine, griseofulvin and tadalafil in supersaturated conditions in different biorelevant media by measuring absorbance (or turbidity) over time.

The media chosen for these assays corresponds to the lowest and highest solubility in both fasted and fed state as stated above for dissolution. The aim was to study if different media compositions and the prandial state have an impact on the ability of these drugs to maintain a supersaturated state.

- **Initial Experiment**

As previously described in Section 2.6.1

- **Precipitation experiment**

As previously described in Section 2.6.2

- **Data analysis**

As previously described in Section 2.6.3

8.3. Results and Discussion

8.3.1 Dissolution Assays

Despite the turbidity of the biorelevant SIF media (Fasted/Fed media 1 and media 2; See Figure 47), the μDiss probes successfully measured drug concentrations of dipyridamole, griseofulvin and tadalafil, *in situ* over the course of 3 hours and without significant point scatter. However, it was found that the point scatter changed depending on the media and on the drug that was being measured. An example of this behaviour is presented in Figure 48 where a comparison of the μDiss dissolution profiles of dipyridamole and aprepitant in the biorelevant SIF media (fasted and fed state) can be seen and where the higher scatter for aprepitant is evident in the fed state. These issues arose by measuring dissolution profiles in complex SIF media and were also reported in a previous study that applied FeSSIF-V2 to measure dissolution [134]. However, all drugs chosen for this study, presented a point scatter that allowed reproducibility in all media tested.

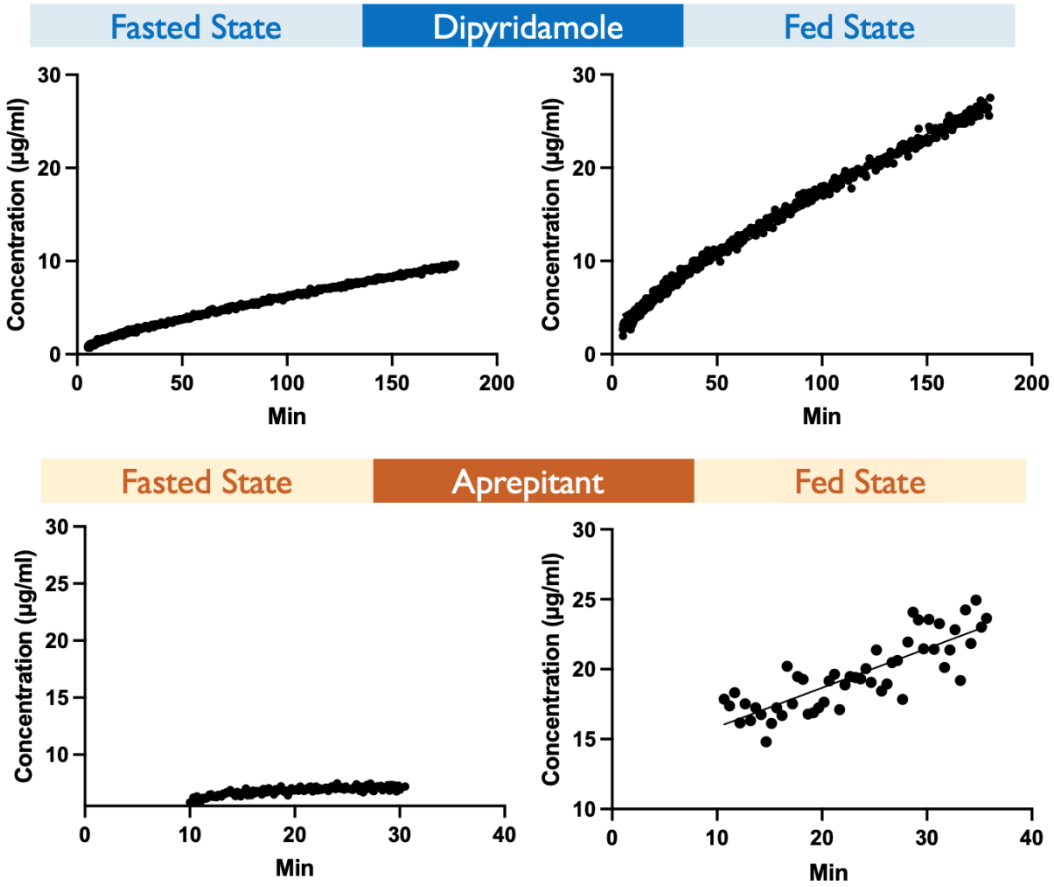


Figure 48. **Dissolution curve scatter comparison between Dipyridamole and Aprepitant in media 2 (fasted and fed state).**

The dissolution profiles of griseofulvin, dipyridamole and tadalafil in the multiple media tested, can be visualised in Figure 49 to Figure 51. Only selected data points are displayed in the dissolution curves; however, all were included in the IDR calculations. The points chosen to be displayed refer to specific sampling intervals: 5 to 15min- every 2 min; 15-30min- every 5 min; 30-70min- every 10 min; 70-160min- every 30 min; last point 180min.

Griseofulvin's dissolution profiles are presented in Figure 49, where it is possible to visualise the changes in its dissolution profile when tested in different SIF media in the fasted and fed states. The dissolution profiles consistently ranked in the same order in both states: Fa/FeSSIF showed the lowest dissolution, followed by media 1 and media 2. This suggests a possible association between the higher concentration of media components such as bile salts, lecithin, and sodium oleate (pH*TAC, Table 6 and Table

7) and improved dissolution. To statistically confirm these observations, a Kruskal-Wallis test was performed to compare the dissolution profiles in the different media within the fasted and fed states. The results revealed significant differences between all three media ($p < 0.0001$) in the fasted and fed state. These findings confirm that the differences observed in the dissolution profiles are statistically significant.

Moreover, FaSSIF and FeSSIF media presented lower dissolution profiles in comparison with the biorelevant SIF media, underlining that for Griseofulvin, there is a dissolution gap not fully captured by using Fa/FeSSIF as reference media. These findings align with previous solubility studies that showed solubility increasing with the amphiphile content of the media (pH*TAC) and in the fed state [129, 248].

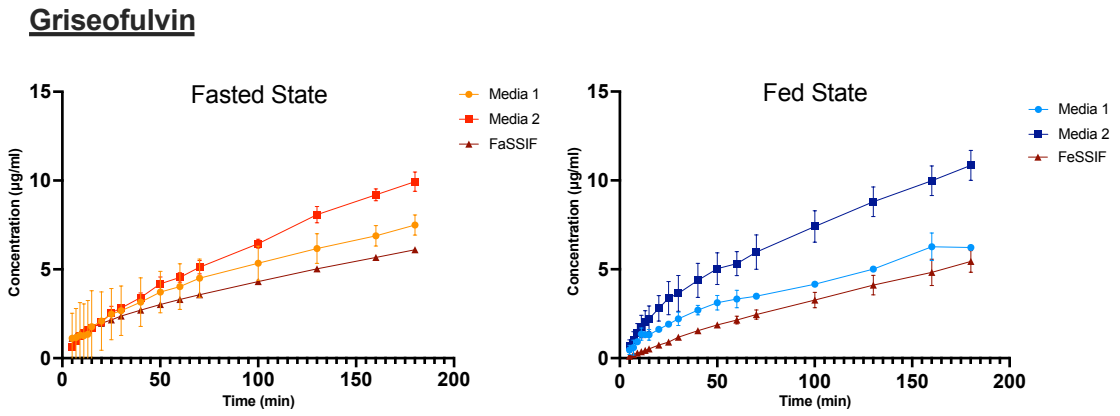


Figure 49. **Dissolution Profiles of Griseofulvin in different SIF media in the fasted and fed state**

Note: Media 1 corresponds to the media with the lowest solubility value in both states. Media 2 corresponds to the highest solubility value. Error bars are a visual representation of the standard deviation between replicates n= 3 (calculated using Prism10).

For dipyridamole (Figure 50), the fasted and fed states present a similar behaviour as griseofulvin, with dissolution profiles ranking: Fa/FeSSIF, Media 1 and Media 2 and portraying the same association between media composition and dissolution profile. Fa/FeSSIF dissolution profiles were not representative of the dissolution space covered by the biorelevant SIF media in both states. Significant differences were observed between all media in the fasted state ($p < 0.0001$), confirming that media composition strongly influences dissolution. In the fed state, most comparisons also revealed

significant differences ($p < 0.0001$), except for Media 1 and FeSSIF, which did not differ significantly.

The dissolution range found in the fasted state was smaller than the dissolution space covered in the fed state a behaviour that is also in line with previous solubility studies [129, 248] where the solubility range for dipyridamole in the fasted state (7.4) was found to be smaller than in the fed state (9.4).

Comparing the fasted and fed states there is a clear increase in the dissolution profiles of dipyridamole in the fed state indicating that the higher concentrations of media components in the fed state seem to have a positive impact on this drug dissolution profile possible relating to its registered food effect.

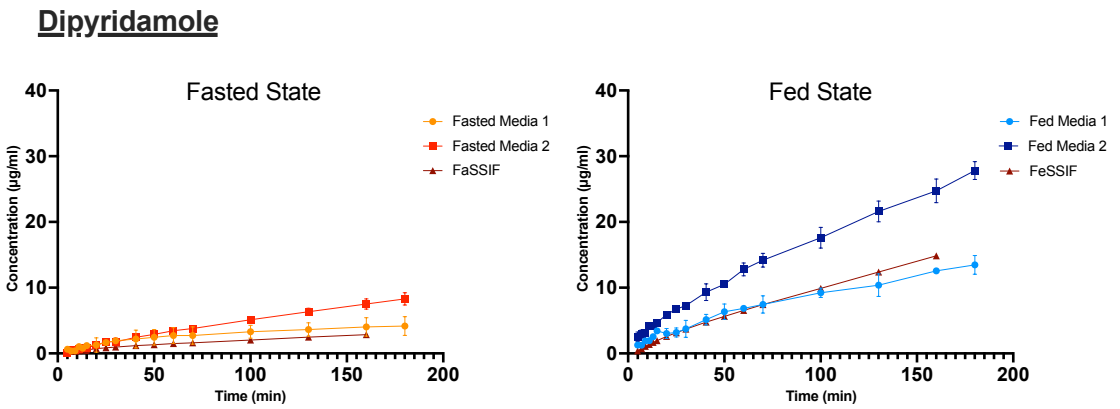


Figure 50. **Dissolution Profiles of Dipyridamole in different SIF media in the fasted and fed state**

Note: Media 1 corresponds to the media with the lowest solubility value in both states. Media 2 corresponds to the highest solubility value. Error bars are a visual representation of the standard deviation between replicates n=3 (calculated using Prism10).

For Tadalafil (Figure 51), the fasted state seems to present a different behaviour than the previous drugs, with FaSSIF media displaying the highest dissolution profile out of the media tested. This behaviour was not seen in the fed state where tadalafil seemed to display a similar behaviour to the previous drugs. Statistical analysis revealed that in the fasted state, there were significant differences between most media ($p < 0.0001$), except between FaSSIF and Media 2, which exhibited similar dissolution behaviours. In the fed state, tadalafil's dissolution behaviour was more aligned with the previous drugs,

with significant differences observed between all media comparisons ($p < 0.0001$) except for FeSSIF and Media 1, which showed no significant difference.

Tadalafil appears to have a tighter dissolution range than the previous drugs indicating that media composition variation has less impact on its dissolution profiles. In previous solubility studies it was reported that tadalafil presents small solubility ranges (Fasted: 3.54 [129], Fed: 4.66 [248]) which also indicates that media variation had less impact on its solubility.

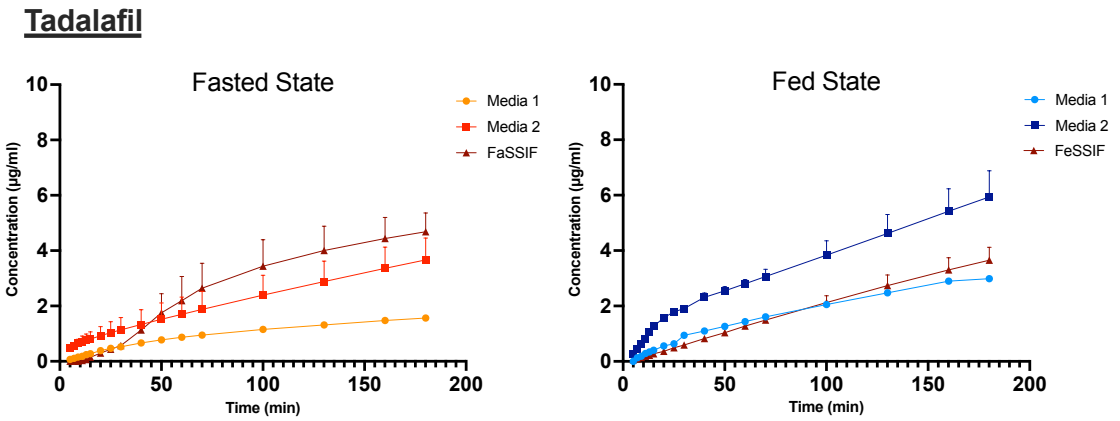


Figure 51. **Dissolution Profiles of Tadalafil in different SIF media in the fasted and fed state**

Note: Media 1 corresponds to the media with the lowest solubility value in both states. Media 2 corresponds to the highest solubility value. Error bars are a visual representation of the standard deviation between replicates n=3 (calculated using Prism10).

The IDR calculated for each drug in the fasted and fed simulated media are displayed in Table 21 along with the respective solubility values.

In the fasted state, the overall trend was that higher IDR values were associated with the media that presented higher solubility in the previous solubility studies. This was evident for dipyridamole and griseofulvin where media 2 resulted in both the highest IDR and highest solubility. Similar behaviour for these two drugs in the fed state where once again media 2 being the highest solubility media also produced the highest IDR. Tadalafil seems to be the exception where in the fasted state FaSSIF presents the highest IDR without being the media with highest solubility. This behaviour highlights that similarly to what was observed in the solubility studies [129, 243-245, 248], some drugs present individualistic behaviours that ultimately limit the prediction of their in

vivo behaviour. Nonetheless, the overall increase in IDR in media with higher solubility indicates a positive correlation between IDR and solubility.

Table 21. **Intrinsic Dissolution Rates for the drugs in study**

Fasted State						
	IDR (μg/min/cm²)			**Solubility (μg/ml)**		
	FaSSIF	Media 1	Media 2	FaSSIF	Media 1	Media 2
Dipyridamole	2.26	2.83	7.02	13.30	16.10	120.50
Griseofulvin	3.83	5.20	7.31	12.00	10.35	24.00
Tadalafil	3.23	1.16	2.21	5.40	3.50	12.40
Fed State						
	IDR (μg/min/cm²)			**Solubility (μg/ml)**		
	FeSSIF	Media 1	Media 2	FeSSIF	Media 1	Media 2
Dipyridamole	12.29	9.16	20.99	76.00	31.40	296.60
Griseofulvin	4.43	4.41	7.51	70.00	29.51	133.39
Tadalafil	2.95	2.16	3.67	16.32	13.16	61.35

Note: Solubility values from previous studies.

In Figure 52, IDR is presented as a function of solubility in the SIF media and the results of Pearson correlation included. Visually, it is clear that higher IDR values are associated with higher solubility values and vice versa. This trend was confirmed by the correlation results where, with the exception of FaSSIF, every SIF media in study presented a positive correlation between IDR and solubility. Positive correlation was found overall for both states (r = 0.80) and individually for the fasted (r = 0.72) and fed state (r = 0.80), although it seems to be stronger in the fed state. This correlation was previously described by the Noyes-Whitney equation (See Chapter 1) that relates dissolution with solubility and diffusion, and where higher solubility results in higher concentrations of dissolved drug in solution and thus increased dissolution rate.

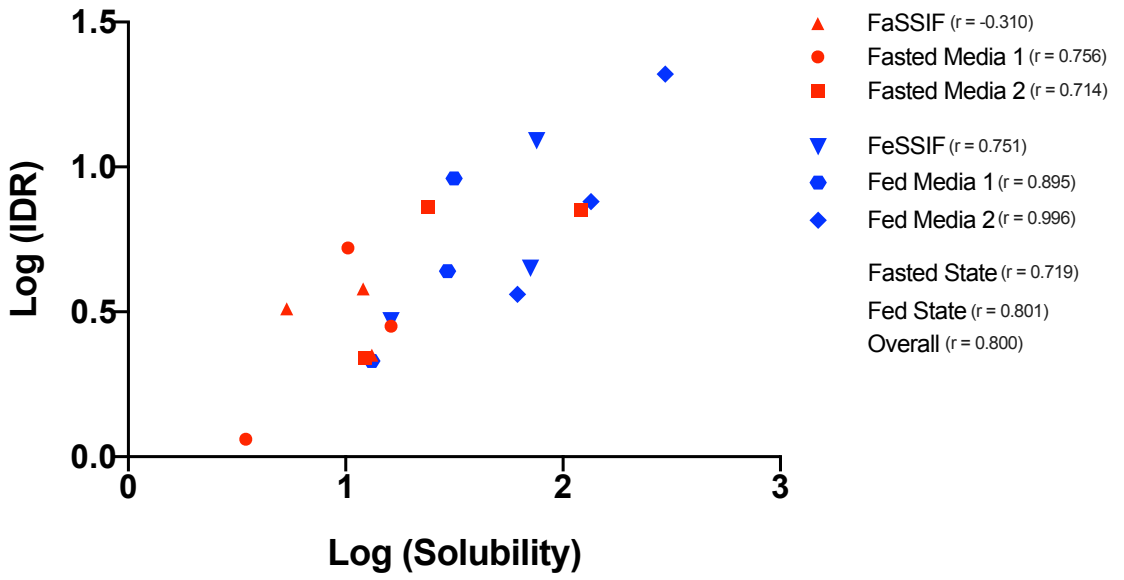


Figure 52. **Intrinsic dissolution rate vs Solubility in the different intestinal media**

Note: Correlation between the IDR and solubility values was performed in Prism 10, using Pearson correlation. Correlation results represented as r. r>0.5 values are correlated; r>0.75 values are strongly correlated.

Another study that applied fasted and fed media to dissolution assays [134], observed that some compounds have their dissolution behaviours limited primarily by the energy required to disrupt their solid crystal lattice. These compounds exhibit limited IDR increases in fed media since the crystal lattice dissociation energy is more critical than micelle partitioning for their dissolution. Griseofulvin and tadalafil were given as examples of compounds with this behaviour by exhibiting limited IDR increases in fed media [134]. Analysing Table 21, griseofulvin's IDR shows a modest increase in the fed state, rising from 3.83 (μg/min/cm^2) in FaSSIF to 4.43 (μg/min/cm^2) in FeSSIF and from 7.31(μg/min/cm^2) to 7.51 (μg/min/cm^2) in Media 2 and Tadalafil also shows only a slight increase in fed conditions, with IDR values changing from 3.23 (μg/min/cm^2) (FaSSIF) to 2.95 (μg/min/cm^2) (FeSSIF) and slightly increasing in Media 2 (from 2.21 to 3.67 μg/min/cm^2). This behaviour seems consistent with the behaviour described in the previous study [134]. Interestingly, further analysis into the melting point of these drugs highlights that it could also play a role in this dissolution behaviour. Griseofulvin and tadalafil, with melting points of 220°C and 302°C respectively, may show limited IDR increases in fed conditions because higher melting points correspond to higher lattice energy, making the crystal structure harder to disrupt.

For compounds with high lipophilicity that might struggle with dissolution due to low water affinity, the fed state media increases significantly their IDR due to the higher micelle volume that aids drug partitioning [134]. This behaviour seems to describe dipyridamole in this study (Table 21) that shows a large increase in IDR in the fed state, particularly in Media 2 with the highest pH*TAC (from 2.26 to 20.99 μg/min/cm²). Dipyridamole seems to exhibit some degree of solvation-limited behaviour, although because of its moderate lipophilicity (log D = 2.88) not to the extreme extent that was registered for very lipophilic compounds (log D > 3) such as danazol [134].

In conclusion, this study demonstrates the significant impact of biorelevant SIF media on the dissolution profiles of dipyridamole, griseofulvin, and tadalafil, revealing dissolution behaviours that are not fully characterised by traditional FaSSIF and FeSSIF media. The results underline the importance of media composition on drug dissolution and highlight a correlation between solubility and IDR, particularly in media with higher amphiphile content. The observed differences between fasted and fed states are consistent with solubility data from previous studies, showing a marked increase in fed state IDR for dipyridamole compared to more moderate differences for griseofulvin and tadalafil. Overall, these findings suggest that exploring dissolution and solubility behaviours using a broader range of biorelevant media can improve understanding of drug-specific responses to fed versus fasted conditions, supporting a more comprehensive approach for predicting in vivo absorption behaviour.

8.3.2 Supersaturation

For each drug, 4 different media were tested, two in the fasted state and two in the fed state, and for each media at least 5 supersaturated concentrations were studied, using the SSPM approach. All media allowed for the measurement of the study drug's time-concentration profile with precipitation successfully detected using μDiss *in situ* probes. It was planned that all initial supersaturation concentrations (CS) tested for each compound started to precipitate within approximately 1 h however, because of time constrictions in some cases that was not possible to achieve.

The time-concentration profiles were plotted as shown in Figure 53 for Tadalafil. These profiles changed depending on the drug and the media in study with some drugs,

like tadalafil in fed media 2 (Figure 53), showing increased point scatter in their profiles after precipitation. This behaviour was previously described as the result of the particles formed during the precipitation process interfering with the UV/Vis measurements [300]. In order to standardise the profiles of the drugs in different media and minimise the impact of the precipitating particles during the measurements, a regression equation was fitted to each curve (plateau followed by one phase decay equation) (See Table 22 for r^2). The regression curves were used to represent and display the propensity of the different compounds to supersaturate in the different media (Figure 54 to Figure 56) and to determine induction time (tind). Table 22 includes the tind values for all drugs in the different media along with the DS that was calculated using the ratio of the initial CS concentration and the equilibrium concentration (Ceq) determined in previous solubility studies.

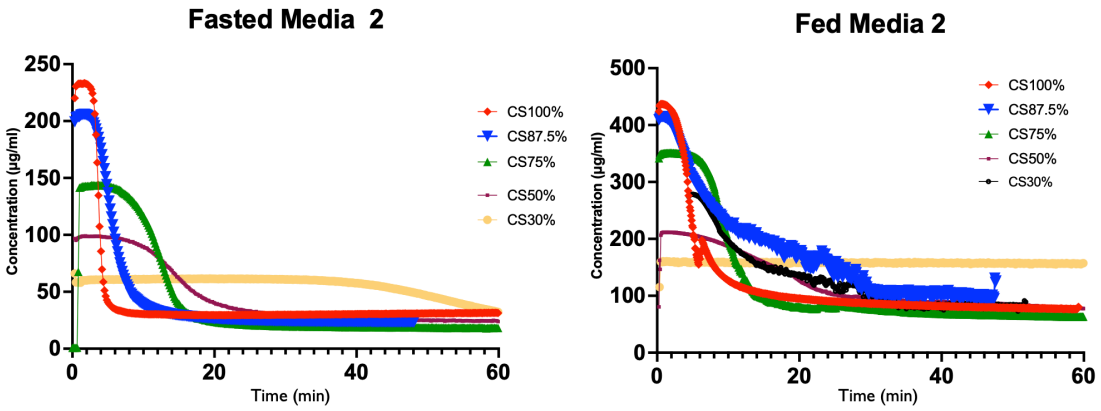


Figure 53. **Time vs Concentration profiles of Tadalafil using 5 DS concentrations**

Note: Cs concentration in µg/ml displayed in Table 22.

For all compounds in different media (Figure 54 to Figure 56), CS100% displayed short tind (0.4-5 min), followed by CS87.5% and CS75% with slightly higher tind times but still portraying fast precipitation of the drugs. Decreasing CS concentrations resulted in longer concentration plateaus before precipitation, with lower CS (e.g., CS50%) exhibiting slower curve decline, which is consistent across all media and drugs. This highlights the balance between achieving high supersaturation and managing stability: while a high CS provides a high DS, it also promotes faster precipitation, whereas lower CS leads to lower DS but prolongs the supersaturation state. That is why testing multiple

initial CS is important since it can help guarantee as high a CS as possible which will reflect in maintain higher DS for a longer period of time.

However, in Figure 54 to Figure 56, it is possible to observe that although the overall trend that higher CS values result in faster precipitation for all drugs, it is clear that when comparing between drugs they present drug-specific behaviours that hamper generalisation. For example, Griseofulvin (Figure 54) precipitated almost immediately at higher concentrations (100%, 85%, 75% CS) in all media for both fasted and fed states (Table 23). Similarly, Felodipine in the fasted state (Figure 56a) showed rapid precipitation at high CS values. However, Tadalafil (Figure 55) in both states, and Felodipine in the fed state (Figure 56b), sustained prolonged supersaturation even at elevated CS. The propensity to supersaturate is thus drug dependent but it also seems to depend on the media that is being tested and its characteristics.

For all drugs examined in this study, Media 2 (with higher amphiphile concentrations) in the fasted state, as well as both fed state media, generally produced higher initial drug concentrations (CS), likely due to greater solubilising capacity and a more gradual precipitation decline. However, this increase in CS did not correlate with a higher DS. Figure 57 to Figure 59 indicate that media with higher solubilising capacity, such as Media 2 and the fed state, do not universally enhance DS. In fact, the fasted state typically achieves higher DS values than the fed state across all drugs (as seen in Figure 57, Figure 58, and Figure 59). For example, tadalafil in fasted Media 1 reaches a DS of 56 at 100% CS, compared to a lower DS of 7 in fed Media 1. Furthermore, SIF media with higher amphiphile concentration (Media 2) consistently exhibits longer induction times than Media 1 (Table 22). For instance, in the fasted state, griseofulvin in Media 2 has a tind of 14.6 minutes at 30% CS, compared to 6.6 minutes in Media 1 at the same CS%. This pattern is consistent across all drugs studied, including tadalafil and felodipine, suggesting that media composition significantly impacts drug supersaturation. Media 2 appears to extend induction times in both prandial states, likely because the higher amphiphile concentration delays precipitation and thus stabilises the supersaturated state. Similarly, comparing fasted and fed states reveals that the fed state generally enhances supersaturation stability, as evidenced by longer induction times (Table 22). For instance, felodipine in fed media has significantly prolonged induction times compared to the fasted state, even at elevated CS levels. This suggests that the fed

state's amphiphilic components, such as bile salts, may inhibit rapid precipitation, thereby promoting supersaturation stability.

A prior study using fasted and fed SIF media [303] found that higher concentrations of bile salts, phospholipids, and lipolysis products in fed media increase drug solubility but can destabilise supersaturation, leading to a quicker onset of precipitation. This contrasts with the findings of the present study, where higher amphiphile concentration in both fed and fasted conditions enhances supersaturation stability, reflected in longer induction times, particularly in the fed state. These discrepancies suggest that supersaturation may not solely depend on solubility-enhancing components but may also be influenced by other media-specific interactions or drug properties. This underlines the complexity of predicting in vivo supersaturation behaviour and highlights the need for careful selection of biorelevant media to simulate the stabilising effects of intestinal components more accurately.

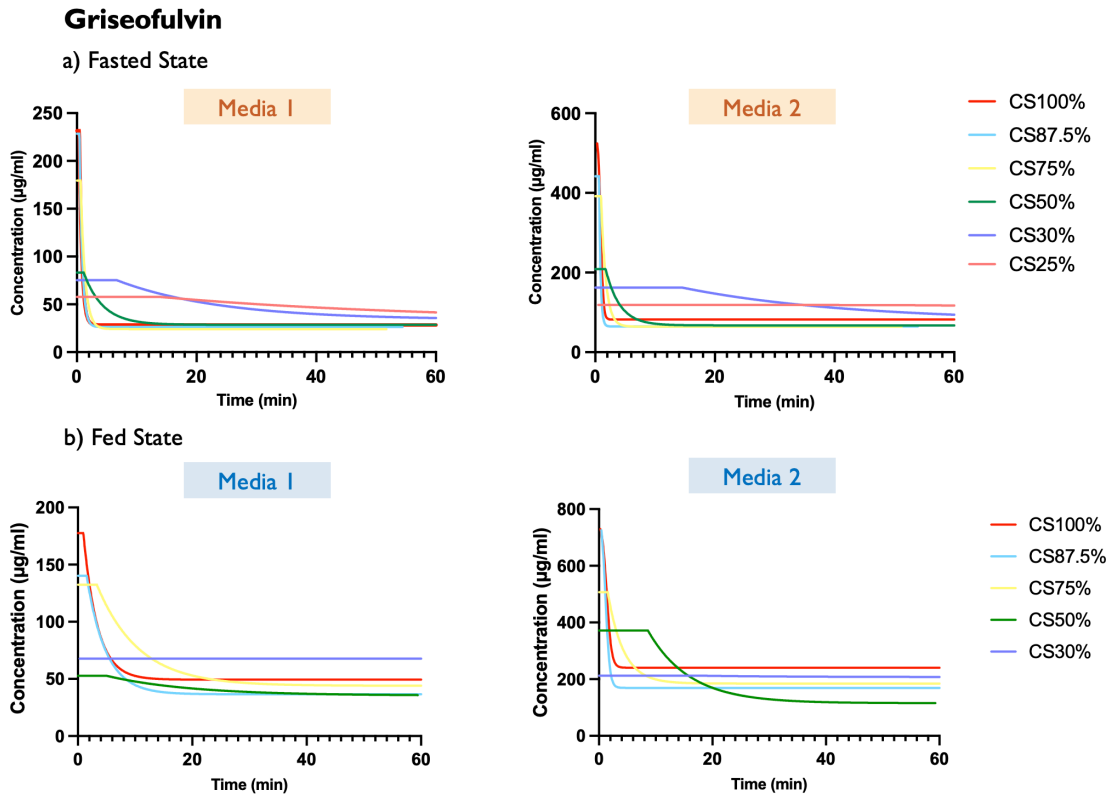


Figure 54. **Time vs Concentration profiles of Griseofulvin in the fasted and fed states**

Note: Plateau followed by one phase decay regression fitted to the available data for each supersaturation concentration (CS). R^2 displayed in Table 22.

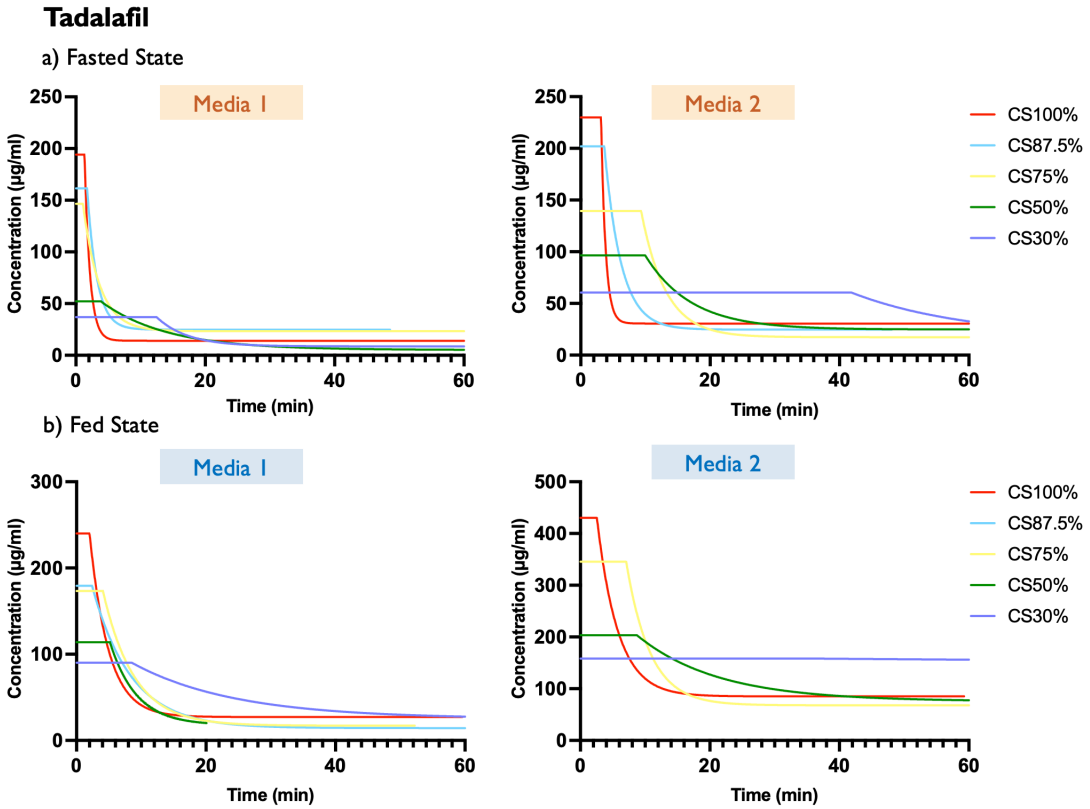


Figure 55. **Time vs Concentration profiles of Tadalafil in the fasted and fed states**

Note: Plateau followed by one phase decay regression fitted to the available data for each supersaturation concentration (CS). R^2 displayed in Table 22.

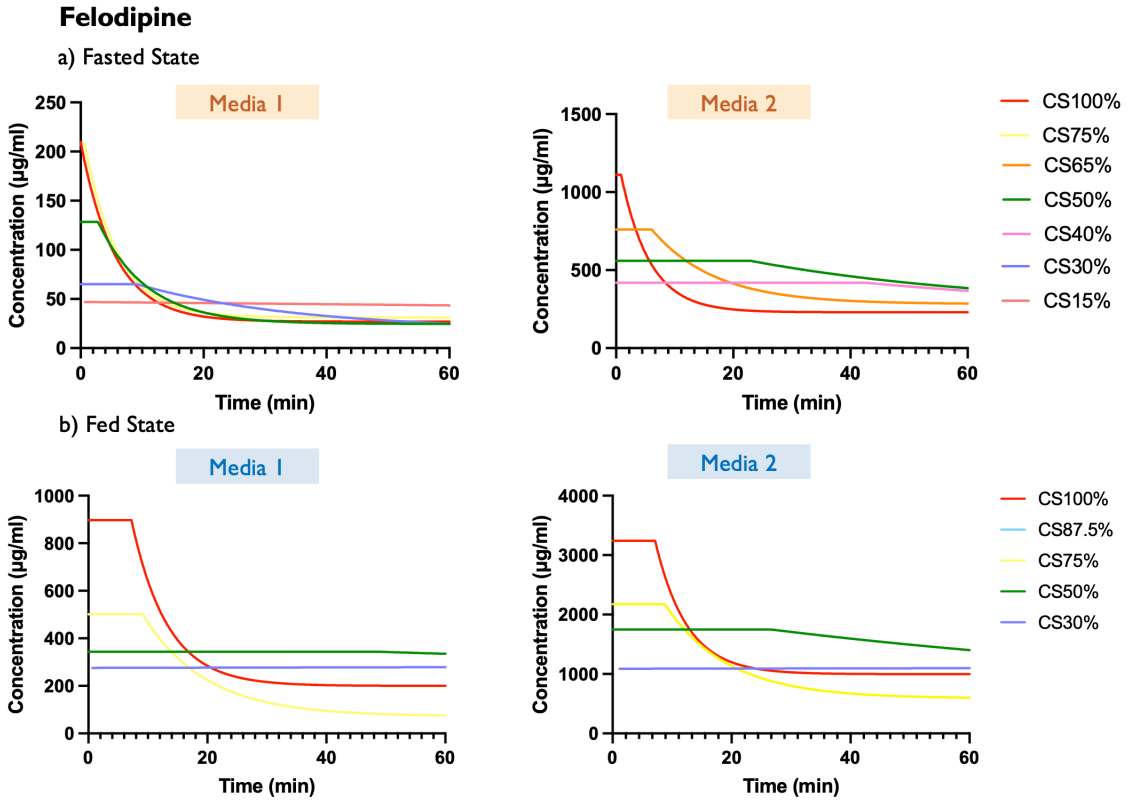


Figure 56. **Time vs Concentration profiles of Felodipine in the fasted and fed states**

Note: Plateau followed by one phase decay regression fitted to the available data for each supersaturation concentration (CS). R^2 displayed in Table 22.

Table 22. **Supersaturation measurements in the fasted and fed state**

Drug	Media	Ceq (µg/ml)	CS %	CS (µg/ml)	DS	t ind (min)	R squared
Griseofulvin Fasted	**1**	10.35	100	231	22.32	0.40	0.85
			88	200	19.32	0.42	0.92
			75	180	17.39	0.62	0.90
			50	83	8.02	1.12	0.95
			30	76	7.34	6.61	0.85
			25	54	5.22	14.01	0.91
Griseofulvin Fasted	**2**	24.00	100	497	20.71	0.46	0.94
			88	440	18.33	0.64	0.97
			75	392	16.33	0.96	0.97
			50	211	8.79	1.73	0.98

Drug	Media	Ceq (μg/ml)	CS %	CS (μg/ml)	DS	t ind (min)	R squared
			30	166	6.92	14.61	0.83
			25	120	5.00	NP	0.93
			100	206	6.98	0.95	0.91
			88	144	4.88	1.53	0.93
Griseofulvin Fed	**1**	29.51	75	135	4.57	3.32	0.94
			50	52	1.76	5.05	0.94
			30	68	2.30	NP	0.91
			100	777	5.83	0.40	0.85
			88	692	5.19	0.62	0.87
Griseofulvin Fed	**2**	133.39	75	511	3.83	1.55	0.92
			50	443	3.32	8.62	0.87
			30	216	1.62	18.10	0.91
			100	196	56.00	1.33	0.84
			88	165	47.14	1.74	0.83
Tadalafil Fasted	**1**	3.50	75	140	40.00	1.09	0.85
			50	55	15.71	3.85	0.88
			30	38	10.86	12.47	0.94
			100	233	18.79	3.14	0.99
			88	205	16.53	3.64	0.99
Tadalafil Fasted	**2**	12.40	75	143	11.53	9.33	0.99
			50	98	7.90	9.96	0.97
			30	62	5.00	41.85	0.97
			100	242	7.16	2.01	0.96
			88	184	5.45	2.40	0.91
Tadalafil Fed	**1**	33.79	75	179	5.30	4.08	0.99
			50	117	3.46	5.15	0.98
			30	91	2.69	8.54	0.98
Tadalafil Fed	**2**	157.54	100	437	2.77	2.55	0.97

Drug	Media	Ceq (μg/ml)	CS %	CS (μg/ml)	DS	t ind (min)	R squared
			75	350	2.22	7.07	0.95
			50	211	1.34	8.72	0.90
			30	170	1.08	36.72	0.94
			100	210	26.92	0.30	0.86
			75	180	23.08	0.64	0.88
Felodipine Fasted	**1**	7.80	50	110.5	14.17	2.82	0.87
			30	70	8.97	9.10	0.84
			15	40	5.13	NP	0.88
			100	1100	7.13	0.85	0.88
			65	770	4.99	6.082	0.92
Felodipine Fasted	**2**	154.30	50	550	3.56	22.94	0.87
			40	420	2.72	42.74	0.83
			100	980	5.86	7.23	0.94
			75	720	4.31	9.12	0.83
Felodipine Fed	**1**	167.16	50	480	2.87	49.04	0.89
			30	291	1.74	NP	0.84
			100	3700	4.90	7.15	0.89
			75	2500	3.31	8.77	0.98
Felodipine Fed	**2**	755.68	50	1850	2.45	26.79	0.93
			30	1100	1.46	NP	0.91

Ceq: equilibrium concentration from previous solubility studies; CS: initial concentration to induce supersaturation; DS: degree of supersaturation ($\frac{Cs}{Ceq}$); tind: induction time: R Squared of the regression curve fitted to the time-concentration profiles.

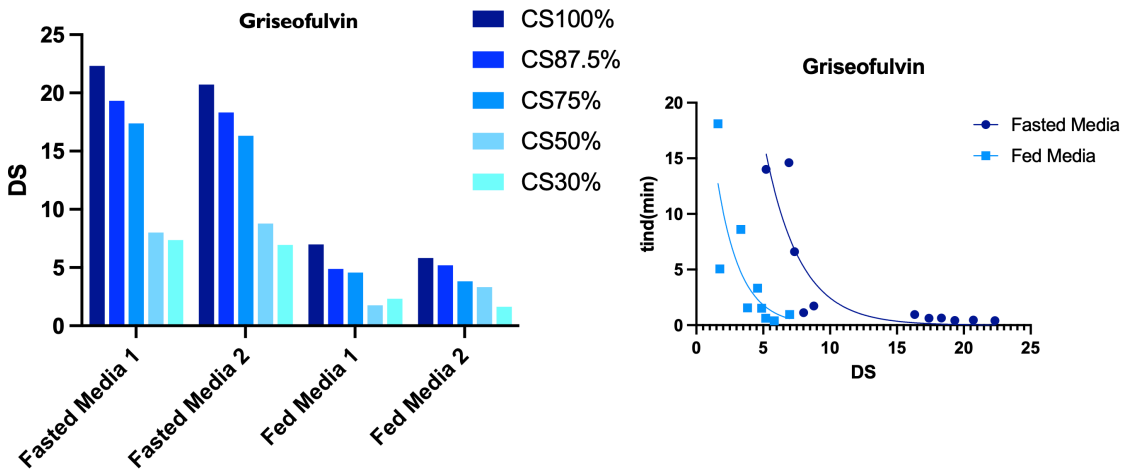


Figure 57. **Degree of Supersaturation and precipitation propensity of Griseofulvin in complex SIF media.**

Note: DS (Degree of supersaturation) and tind (induction time).

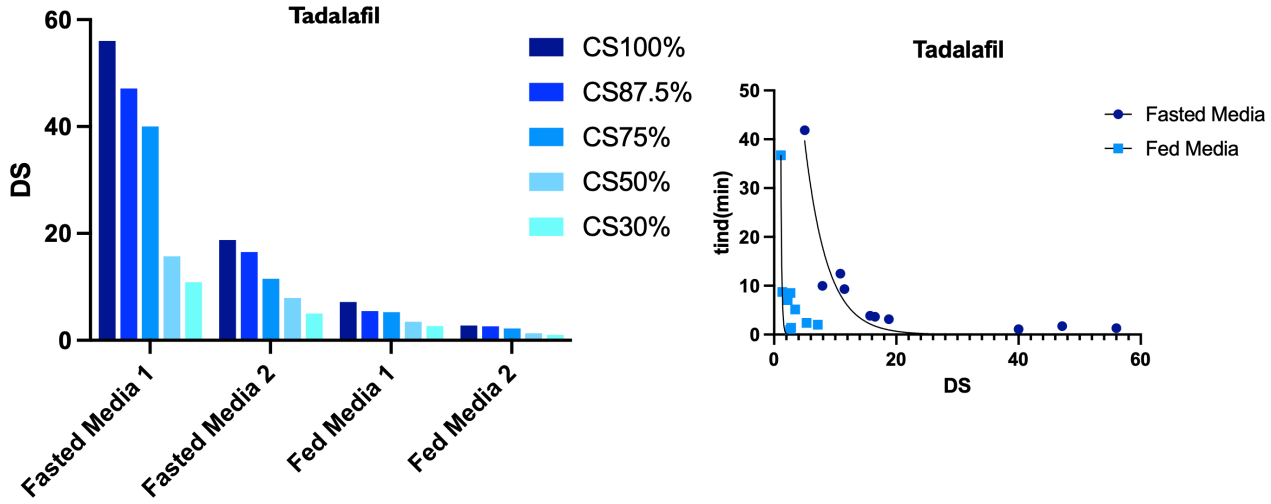


Figure 58. **Degree of Supersaturation and precipitation propensity of Tadalafil in complex SIF media.**

Note: DS (Degree of supersaturation) and tind (induction time).

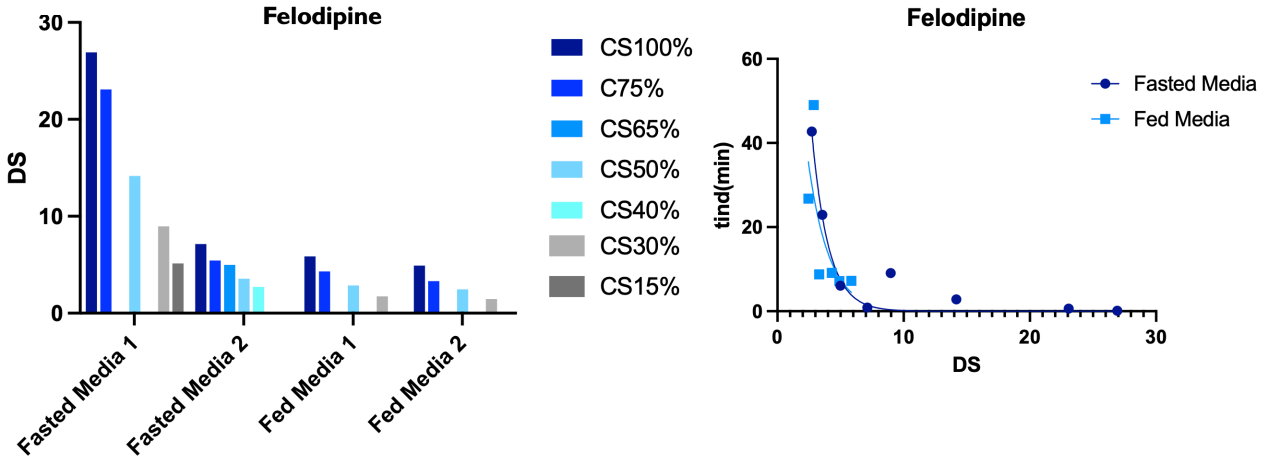


Figure 59. **Degree of Supersaturation and precipitation propensity of Felodipine in complex SIF media.**

Note: DS (Degree of supersaturation) and tind (induction time).

8.4. Conclusion

Dissolution Studies

This study explored the dissolution profiles of dipyridamole, griseofulvin, and tadalafil in biorelevant fasted and fed state SIF media, highlighting the impact of media composition on dissolution. Despite the turbidity of the media, the use of µDiss probes allowed for precise *in situ* measurements over three hours, showing reproducible dissolution profiles across all media, albeit with some variation in scatter depending on the drug and media composition. Dissolution profiles and IDR were strongly influenced by amphiphile concentration (pH*TAC), particularly for dipyridamole and griseofulvin. These drugs displayed larger dissolution ranges in the fed state, aligning with solubility trends observed in previous studies. Tadalafil showed narrower dissolution ranges, indicating less sensitivity to media variation particularly in the fasted state. The calculated IDR correlated positively with solubility in most media, further supporting the relationship described by the Noyes-Whitney equation. However, individual drug behaviours such as tadalafil's unique fasted state dissolution underscore the limitations of general predictive models.

Overall, the dissolution study highlights the importance of using biorelevant SIF media to capture a broader range of dissolution behaviours compared to traditional FaSSIF and FeSSIF media. Exploring these dissolution behaviours provides greater insight into drug-specific responses to fasted and fed conditions, ultimately enhancing biopharmaceutical understanding and formulation design.

Supersaturation and Precipitation

This study focused on the supersaturation behaviour of griseofulvin, tadalafil, and felodipine across four biorelevant media—two in the fasted state and two in the fed state—using the supersaturation and precipitation monitoring SSPM approach. Supersaturation behaviour was successfully measured using µDiss probes, with time-concentration profiles analysed to determine induction times (tind) and DS. Higher CS led to rapid precipitation, while lower CS prolonged supersaturation, reflecting a balance between stability and solubility enhancement.

Media composition strongly influenced supersaturation behaviours. Media 2, characterised by higher amphiphile concentrations, and the fed state media consistently showed greater solubilising capacity and extended tind values. However, the fed state did not universally increase DS; fasted media often achieved higher DS across all drugs. For instance, tadalafil displayed a DS of 56 in fasted Media 1 compared to a much lower DS of 7.16 in fed Media 1. These findings underscore that high solubilising capacity does not directly correlate with supersaturation enhancement. Induction times were generally longer in fed media, reflecting stabilisation by amphiphilic components such as bile salts. Media 2 consistently delayed precipitation compared to Media 1 in both prandial states, further underscoring the impact of amphiphile concentration on supersaturation stability.

Interestingly, these findings deviate from prior studies, which suggested that high bile salt and amphiphile concentrations in fed media could destabilise supersaturation by accelerating precipitation. In contrast, the present study found that higher amphiphile concentrations stabilised supersaturation, particularly in fed conditions. These results highlight the complexity of supersaturation experiments, driven by drug-specific behaviours and nuanced media-drug interactions, and reinforce the necessity of

carefully selecting biorelevant media to model in vivo supersaturation dynamics accurately.

Chapter 9

Research Outcomes and Future Work

9.1 Thesis conclusion

This thesis serves as the latest chapter in over a decade of research efforts at the University of Strathclyde, initiated during the Orbito project, to develop a more biorelevant approach to measure *in vitro* solubility. Building on the knowledge gained in the first DoE experiments where the role of media components on solubility was underscored and later reduced DoE approaches that tried to reduce the number of experiments required to still maintain a relevant solubility range, to the multidimensional analysis of HIF and the media recipes that describe component variation in real-world populations. This work consolidates this previous knowledge and introduces an innovative *in vitro* method for measuring solubility and other biopharmaceutical parameters in the fed state, contributing to the advancement of predictive tools in pharmaceutical science.

The main aim was to apply the fed biorelevant media system to study drug solubility in the fed environment and both fasted and fed biorelevant systems to dissolution and supersaturations experiments.

The first objective (Chapter 3) was to compare the equilibrium solubility of drugs using this fed state biorelevant media derived from multidimensional analysis versus the previous fed DoE approaches to validate the feasibility of the new method. The results indicated that the 9-media system did not fully replicate the solubility data of the fed 92-point DoE system, because the initial fed 92-point DoE applies excessive media component concentration ranges compared to the 9 media system, due its statical foundation. The elimination of the outlier media compositions improved the statistical agreement between the systems and highlighted that the 9-media biorelevant system

offers a more realistic estimate of fed state solubility than the larger scale DoE approaches.

The second objective of this study (Chapter 4) was to apply a fed state solubility range to the DCS grid, a novel approach in the field, and assess the solubility behaviour across a population using a solubility frequency distribution. The nine fed media recipes provided reliable equilibrium solubility measurements, aligning with published fed HIF and SIF values. These measurements, along with the SLAD values, offer valuable data for risk assessments and QbD strategies allowing to determine best- and worst case scenarios of drug solubility and detect population solubility variations that would not be possible with single measurements. A comparison with fasted state solubility revealed notable differences, suggesting that combining fasted and fed data from these biorelevant systems could be used as a tool to improve in vivo prediction accuracy. This approach, akin to the fasted media system, merited further investigation to expand its drug application, link *in vitro* solubility with drug characteristics (later studied in Chapter 5) and explore fasted vs. fed solubility behaviours in more detail (later studied in Chapter 7).

The third objective (Chapter 5) of this study was to examine the solubility behaviour of all 24 drugs (Table 8) to identify patterns that could reduce the number of simulated media measurements required to establish a fed state solubility range, aiding early drug development with limited API material. This study found that the solubility trends observed were consistent with previous studies, with acidic drugs (category 1) showing pH-dependent solubility, and basic and neutral drugs (category 2) exhibiting solubility influenced by pH × TAC. Some drugs showed minimal solubility variation (category 3), while others displayed unusual behaviour (category 4). In most cases, two media (Media 1 and Media 2) successfully captured the fed solubility range, highlighting the utility of this method in drug development. Combined with fasted state data, this approach revealed promising potential for defining solubility envelopes and warranted further investigation to determine how well this media system correlated with HIF literature data.

The fourth objective (Chapter 6) was to establish an *in vitro*/in vivo intestinal solubility correlation by comparing Fa/Fe9SIF solubility data for various drugs with published Fa/FeHIF solubility values. The results indicated no significant solubility

difference between Fa/Fe9SIF and Fa/FeHIF, suggesting that Fa/Fe9SIF could be considered bioequivalent to Fa/FeHIF. A novel solubility boundary analysis supported this conclusion, reinforcing the potential of Fa/Fe9SIF to model *in vitro* solubility for various populations and patient groups. This bioequivalent solubility range could be applied to improve PBPK and in silico models, reducing the need for in vivo testing.

The fifth objective (Chapter 7) developed a new predictive framework for assessing food effects on drug absorption by combining solubility measurements in fasted and fed biorelevant media with SLAD calculations. Acidic drugs displayed minimal solubility differences due to pH dependence, while basic and neutral drugs exhibited significant fed state solubility increases, driven by TAC. Correlating these solubility shifts with in vivo bioavailability revealed a strong predictive link, particularly for neutral drugs ($r = 0.810$). A decision tree, incorporating solubility, Dose/SLAD thresholds, and metabolism, achieved 83% prediction accuracy, highlighting the potential of this approach as a tool for anticipating food effects in drug development. Future work should expand datasets and integrate additional pharmacokinetic factors to enhance reliability.

The sixth objective (Chapter 8) was to study the impact of media changes on the drug's IDR and correlate these findings with previous solubility data. It also sought to provide a detailed analysis of supersaturation behaviour under fasted and fed conditions using biorelevant SIF media. This study emphasised the impact of media composition on dissolution and supersaturation behaviours of selected drugs, highlighting drug-specific responses and the importance of biorelevant SIF media in capturing these nuances. The dissolution profiles demonstrated reproducibility and aligned with solubility trends. Supersaturation studies showed that higher amphiphile concentrations, particularly in fed media, stabilised supersaturation by extending induction times. However, a high solubilising capacity did not directly correlate with increased supersaturation, as the fed state did not universally lead to higher DS. Discrepancies with previous literature findings stress the complexity of drug-media interactions and the importance of continuing to apply more complex media to the study of drug precipitation.

Overall, this thesis advances biopharmaceutics by introducing a novel fed state biorelevant system that builds upon a decade of research to measure drug solubility more accurately. It highlights the utility of examining intestinal fluids as a multidimensional system, providing critical insights into drug behaviour under fasted

and fed conditions that are not detectable with single media approaches. The approach presented enables the generation of refined and efficient simulated fluids, where just four media (two for fasted and two for fed states) can encompass best- and worst case solubility scenarios that could easily be applied as a tool in drug development. Further exploration of intestinal fluid composition and properties could expand this multidimensional analysis that has the potential to enhance predictive models and drug development strategies.

9.2 Future work

This thesis introduced a novel fed state biorelevant media system and explored its applications in solubility, dissolution, and supersaturation studies under both fasted and fed conditions. While the developed methods significantly enhanced the understanding of the impact of fed conditions on drug solubility and dissolution, there remain areas for further investigation. Future work could involve:

1. **Increase the data set of drugs available:** This study examined 24 drugs, but expanding this number would allow the inclusion of compounds with diverse behaviours, enhancing the robustness of this solubility systems. To improve the robustness of the food effect prediction model, future work should include additional drugs with well-documented food effects, or the absence thereof, in the literature. Moreover, incorporating drugs with established human intestinal solubility values would strengthen the correlation between the nine media system and in vivo solubility data, further validating its applicability for predictive biopharmaceutical assessments.
2. **Media Optimisation**: The nine media fed state system could be further refined by incorporating additional components, such as monoglycerides and other bile salt species, to enhance its biorelevance and better mimic intestinal conditions. Re-analysing the original dataset used to develop the system through an updated multidimensional analysis could also identify additional key factors to include. Comparing solubility data from the current and modified systems would provide valuable insights into the impact of these new components on drug solubility.
3. **Improve the fed state collection study**: The fed state media applied in this study derives from Ensure Plus which presents certain limitations due to its nature as a liquid nutritional supplement rather than actual solid food. Ensure Plus is not digested in the same way as solid food, leading to reduced interindividual variability compared to the complex digestion of a high-fat FDA-recommended breakfast. When consuming solid food, individuals exhibit variable gastric emptying rates, enzyme activity, and bile responses, all of

which influence drug solubility and absorption differently. Future work could involve perform a new collection of HIF to perform a multidimensional analysis but this time administer an FDA breakfast and analyse the composition of the intestinal media in comparison with the previous Ensure study.

4. **Special Populations and Disease states:** The multidimensional analysis has significant potential for application in special populations, such as the elderly and children, whose gastrointestinal physiology differs from that of healthy adults. By analysing HIF specific to these groups and applying multidimensional analysis, biorelevant media could be tailored to more accurately reflect their unique physiological conditions, enabling better predictions of drug solubility and absorption. This methodology could also be extended to patients with intestinal diseases, where altered pH, enzyme activity, and bile salt concentrations significantly impact drug behaviour. Such tailored approaches would enhance precision in drug formulation and therapeutic strategies.
5. **In Vivo Correlation with PBPK Models**: Integrating biorelevant solubility, dissolution, and supersaturation data into PBPK models offers an opportunity to refine predictions of bioavailability and drug absorption in both fed and fasted states. These models could simulate complex interindividual variability and food effects, filling gaps identified in this study. Further, PBPK models could expand the application of biorelevant systems to special populations and disease states, allowing for virtual clinical trials to optimise formulations and dosing regimens.
6. **Supersaturation and Precipitation**: Applying the fasted and fed biorelevant systems to precipitation studies underscored the highly drug- and media-specific nature of these phenomena. Expanding the dataset by testing a larger variety of drugs could help validate the unique behaviours observed in this study. Additionally, analysing the characteristics of precipitated crystals and their interactions with media components—such as amphiphiles, bile salts, and pH—would provide deeper insights into the mechanisms governing precipitation and recrystallisation.

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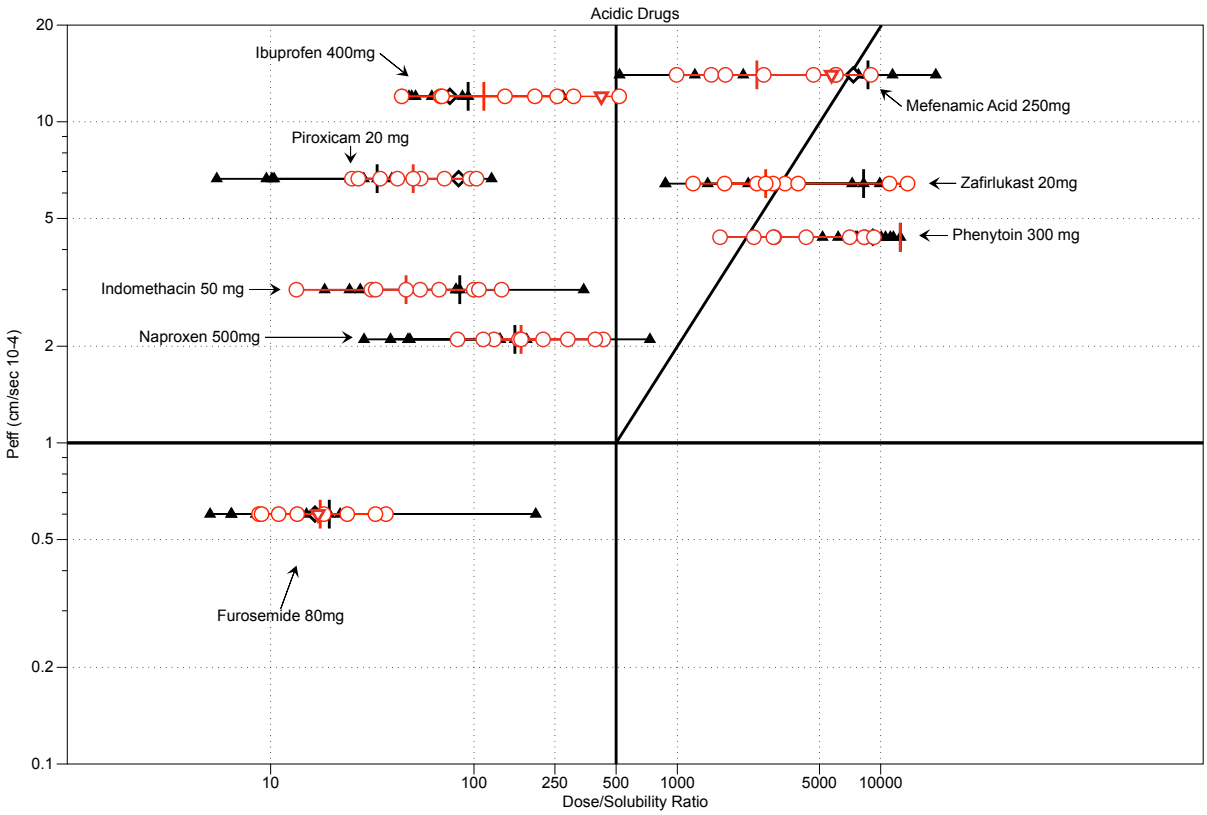
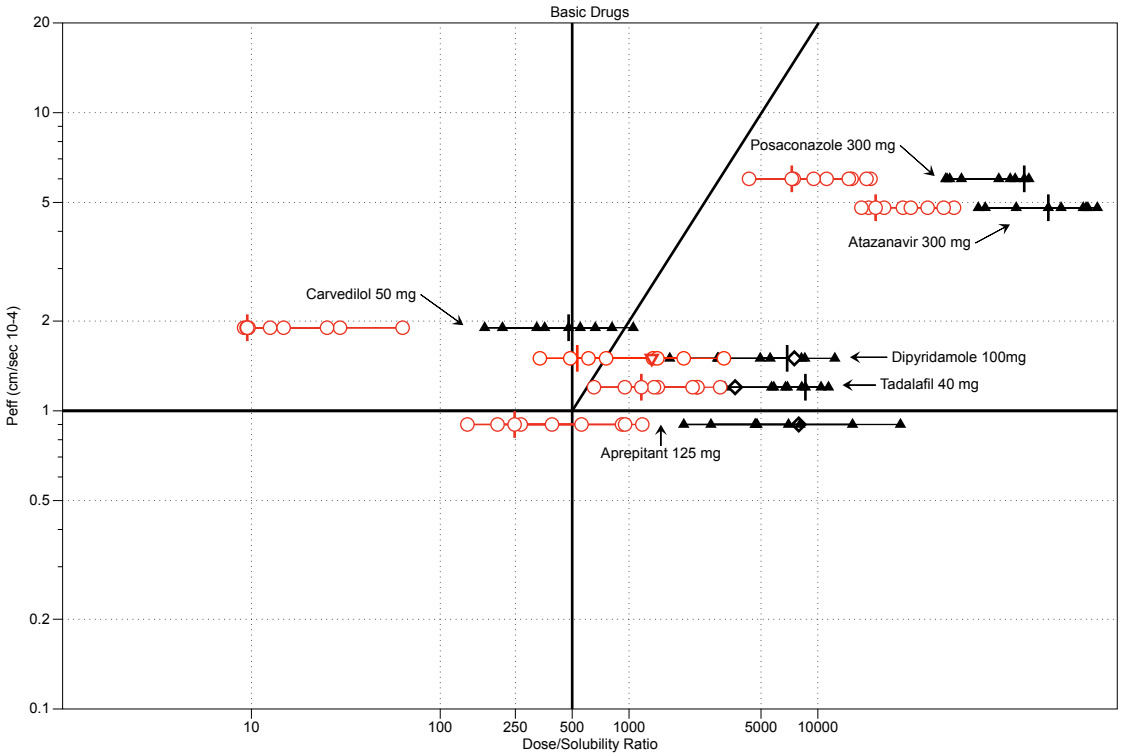
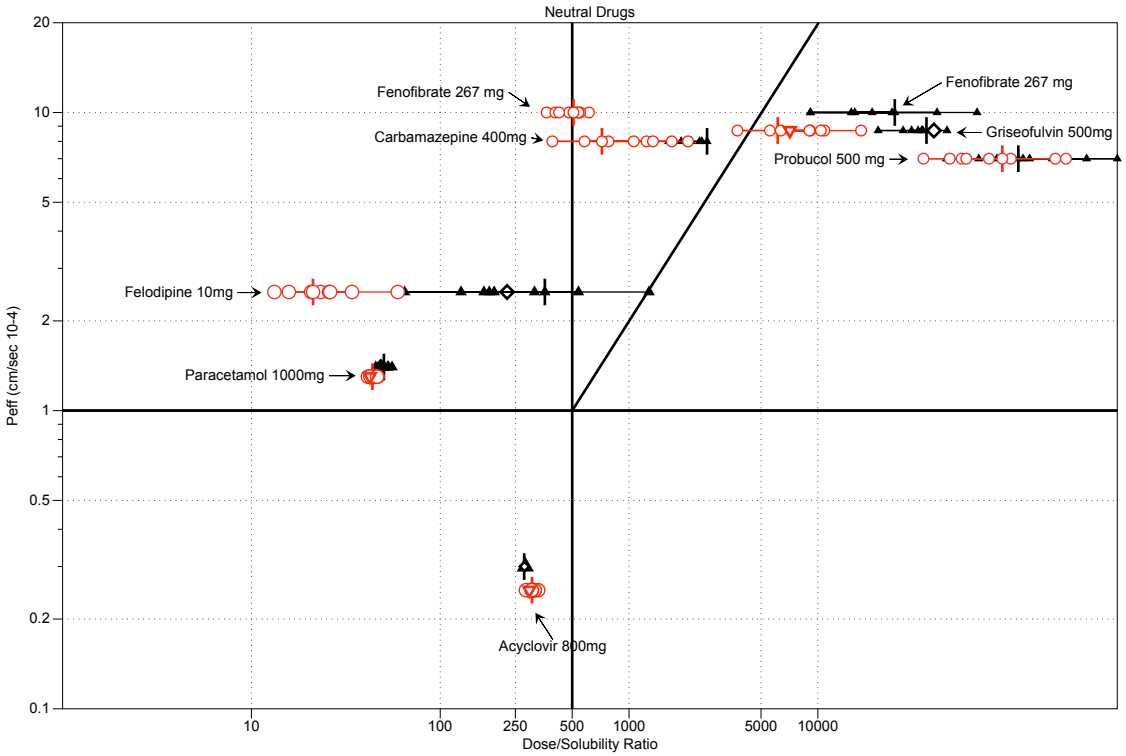
Appendix A

Fasted and Fed Biorelevant Systems on Developability Classification System Grid.

Note: Δ Fasted Nine Media data points, ○ Fed Nine Media data points; Individual drugs and doses as labelled. Each point mean n = 3.




Basic Drugs
Peff (cm/sec 10-4)
20
10
5
2
1
0.5
0.2
0.1
Posaconazole 300 mg
Atazanavir 300 mg
Carvedilol 50 mg
Dipyridamole 100mg
Tadalafil 40 mg
Aprepitant 125 mg
10
100
250
500
1000
5000
10000
Dose/Solubility Ratio


Neutral Drugs
Peff (cm/sec 10-4)
20
10
5
2
1
0.5
0.2
0.1
Fenofibrate 267 mg
Carbamazepine 400mg
Fenofibrate 267 mg
Griseofulvin 500mg
Probucol 500 mg
Felodipine 10mg
Paracetamol 1000mg
Acyclovir 800mg
10
100
250
500
1000
5000
10000
Dose/Solubility Ratio