In vitro Release Testing as an Alternative to Establishing Bioequivalence of Drug Products *in vivo*

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Acknowledgements and Dedication

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Except where stated otherwise by reference or acknowledgment, the work presented was generated by myself under the supervision of my advisor Prof. Dr. Jennifer Dressman during my doctoral studies. A detailed explanation of my personal contributions and contributions of co-authors to the peer-reviewed publications this thesis is based on is provided in the Appendix (A.1.2.).

Whenever a figure, table or text is identical to a previous publication, it is stated explicitly in the thesis and copyright permission and/or co-author agreement has been obtained.

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Exact reference regarding the original journal publication of the figures and tables is given in the description of the specific figure or table.

Contents

List of Abbreviations

AAFE	Absolute Average Fold Error
ACAT	Advanced Compartmental and Transit
A_n	Absorption Number
API	Active Pharmaceutical Ingredient
AUC	Area Under the Concentration-Time Curve
BA	Bioavailability
BCS	Biopharmaceutics Classification System
BDDCS	Biopharmaceutics Drug Disposition and Classification System
BE	Bioequivalence
Caco-2	Cell Line of Heterogeneous Human Epithelial Colorectal Adenocarcinoma Cells
CFR	Code of Federal Regulations
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
C_{max}	Maximum Plasma Concentration
CMS	Concerned Member State
CP	Centralized Procedure
CV	Coefficient of Variation
D_0	Dose Number
DCP	Decentralized Procedure
DCS	Developability Classification System
D_n	Dissolution Number
DS	Dose/Solubility-Ratio
EC	European Commission
EHC	Enterohepatic Recirculation
EMA	European Medicines Agency
EML	Essential Medicines List
EU	European Union
f_a	Fraction Absorbed from the Intestinal Lumen
FaSSGF	Fasted State Simulated Gastric Fluid
FaSSIF	Fasted State Simulated Intestinal Fluid
FDA	United States Food and Drug Administration
FDC	Fixed-Dose Combination
FeSSGF	Fed State Simulated Gastric Fluid
FeSSIF	Fed State Simulated Intestinal Fluid
FIP	International Pharmaceutical Federation
GIT	Gastrointestinal Tract
GSE	General Solubility Equation
hPEPT	Human Peptide Transporter
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl Methylcellulose
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IR	Immediate Release
IVIVC	*in-vitro in-vivo* Correlation
LD_{50}	Lethal dose for 50% of tested population
LogP	Common Logarithm of the Octanol-Water Distribution Coefficient
MA	Market Authorization
MDCK	*Madin-Darby* Canine Kidney Cells
MMC	Migrating Myoelectric Complex
MR	Modified Release
MRI	Magnetic Resonance Imaging
MRP	Mutual Recognition Procedure
NTI	Narrow Therapeutic Index
OrBiTo	EU funded Initiative for *Innovative Tools for Oral Biopharmaceutics*
PAMPA	Parallel Artificial Membrane Permeability Assay
P_{app}	Apparent Permeability
PAR	Public Assessment Report
PBBM	Physiologically Based Biopharmaceutic Modelling
PBPK	Physiologically Based Pharmacokinetic Modelling
PE	Pharmaceutical Entrepreneur
Ph. Eur.	European Pharmacopoeia

PK	Pharmacokinetic
$pK_{a/b}$	Negative Common Logarithm of the Acid (**a**) or Base (**b**) Dissociation Constant
PQTm	Prequalification Team for Medicines
QC	Quality Control
RD	Rapidly Dissolving
rDCS	Refined Developability Classification System
RMS	Reference Member State
RPM	Revolutions per Minute
$SGF_{(sp)}$	Simulated Gastric Fluid (*sine* Pepsin)
$SIF_{(sp)}$	Simulated Intestinal Fluid (*sine* Pancreatin)
SLS	Sodium Lauryl Sulfate
SODF	Solid Oral Dosage Form
Surfactant	Surface active Agent
t_{Disint}	Time Until Complete Disintegration
THz-TDS	Terahertz Time-Domain Spectroscopy
TIM	*TNO* (Gastro-)Intestinal Model
t_{max}	Time of Maximum Observed Plasma Concentration
TPI	Terahertz Pulsed Imaging
USP	United States Pharmacopoeia
VBE	Virtual Bioequivalence
VRD	Very Rapidly Dissolving
WHO	World Health Organization

List of Symbols

$\mathbf{A}$	Surface Area
$\mathbf{C}$	Concentration of a Solute in a Solvent
$\mathbf{C_s}$	Saturation Concentration of a Solute in a Specific Solvent
$\mathbf{d}$	Infinitesimal Difference (Differential)
$\mathbf{D}$	Diffusion Coefficient
$\mathbf{f_2}$	Dimensionless Similarity Factor
$\mathbf{h}$	Thickness of the Hydrodynamic Diffusion Layer
$\mathbf{M_0}$	Initial Solid Mass Available for Dissolution
$\mathbf{M_n}$	Cumulative Fraction of Dissolved Mass at Time $\mathbf{t_n}$
$\mathbf{M_s}$	Solid Mass Available for Dissolution
$\mathbf{n}$	Total Number of Individual Time Points Used for Numerical Calculation of Disintegrated Mass at Time $\mathbf{t_n}$
$\mathbf{n_D}$	Sample Size (Number of Time Point Comparisons in $\mathbf{f_2}$-Test)
$\mathbf{r_0}$	Initial Particle Radius
$\mathbf{R_t}$	Amount [%] Released from the Reference Drug Product at Time $\mathbf{t}$
$\mathbf{t}$	Time
$\mathbf{T_t}$	Amount [%] Released from the Test Drug Product at Time $\mathbf{t}$
$\mathbf{V}$	Fluid Volume
$\mathbf{w_i}$	Fraction of Disintegrated Mass at Time $\mathbf{t_i}$
$\mathbf{z}$	Hybrid Dissolution Constant [Comprised of $3\mathbf{D}/(\mathbf{h}\cdot\boldsymbol{\rho}\cdot\mathbf{r_0})$]
$\boldsymbol{\beta}$	Type II Error Probability (False-Negative Rate)
$\boldsymbol{\rho}$	Particle Density

1. Introduction

1.1. Background

Generic medicinal products are defined by the *European Medicines Agency* (EMA) as drug products that, when compared to a designated reference medicinal product, are essentially the same with respect to certain aspects such as the active pharmaceutical ingredient(s) (API), dosage strength, route of administration, medical indication and quality standards during manufacture[1–3]. They may, however, differ in their outer appearance (name, packaging and appearance of the dosage form) and, most importantly, in their qualitative and quantitative composition regarding the inactive ingredients (excipients) used during manufacture[2].

In order to ensure their therapeutic equivalence and thus interchangeability in public health-care, bioavailability studies that compare the pharmacokinetics of both the generic and the comparator drug product in terms of rate and extent of absorption, with the aim of demonstrating bioequivalence (BE), are required[1]. Combined with the requirement of meeting equal quality standards in manufacture, demonstration of BE serves as a surrogate for therapeutic equivalence of generic drug products, which would otherwise have to be proven in lengthy and laborious clinical safety and efficacy trials[1].

For highly soluble APIs formulated as immediate release (IR) solid oral dosage forms (SODF), the regulatory burden associated with applying for generic approval can be reduced even further. For such APIs the demonstration of BE *in vivo* may be waived in favour of comparative release testing *in vitro* in a procedure named *Biopharmaceutics Classification System (BCS) based biowaiver*[3–6]. Application of the BCS-based biowaiver avoids unnecessary testing in humans, saves the cost of a BE study (approximately 250,000$[7]), and more directly assesses a key quality aspect of solid oral dosage forms: the dissolution performance[8].

In addition to potential savings for the pharmaceutical entrepreneur (PE) when applying a BCS-based biowaiver, widespread availability of generic medicinal drug products also contributes extensively to reducing expenses in public healthcare: in Germany, generics and biosimilars accounted for 78% of the defined daily doses prescribed in 2018, whereas they accounted for only 23% of the total sales volume of medicines and 9.3% of the total public healthcare budget[9]. Furthermore, when more than one PE participated in discount contracts with public health insurance companies for preferred distribution, lack of availability of the drug product was reported fewer times compared to contract models which granted exclusivity to one PE[10].

Apart from occasional short-term supply shortages of certain drug products, access to adequate healthcare and medicinal products is generally well assured in developed countries such as Germany. However, global healthcare accessibility and especially the availability of drug products of sufficient pharmaceutical quality listed on the *World Health Organization*

(WHO) *Essential Medicines List* (EML)[11] still leaves much room for improvement. The WHO estimated in 2011 that adequate access to essential medicines was guaranteed for less than two thirds of the global population[12,13], and several authors attribute this supply gap in developing countries to the high cost of medicines[14] in addition to insufficient funding and infrastructure for regulatory quality control (QC) and operational health care systems[15,16].

Availability of generic drug products of essential medicines is a key aspect in lowering the treatment cost of high-burden diseases in developing countries. Approximately 90% of the APIs listed on the WHO EML are patent-free[17], and several patented essential medicines used for the treatment of HIV/AIDS, hepatitis C and tuberculosis are licensed to international organisations such as the *Medicines Patent Pool*[18], allowing them to be manufactured and distributed at lower prices in low- and middle-income countries.

Addressing infrastructural issues, the WHO collaborates with national authorities and has released several guidance documents (including the 21st EML[11] and guidance on *in vitro* equivalence testing[5]) with the aim of assisting in establishing national drug policies in countries that struggle to provide adequate access to public healthcare[19]. As part of their strategy, the WHO drafted a tabular overview of the solubility and permeability classification of essential medicines in 2006[20] that was intended to be continually revised[21], with the aim of compiling potential candidates for a BCS-based biowaiver among the essential medicines.

Supporting the WHO initiative, the *International Pharmaceutical Federation* (FIP) special interest group on BCS and Biowaiver publishes *Biowaiver Monographs* that summarize publicly available data relevant to a possible BCS-based biowaiver of APIs (with a focus on essential medicines) and provide a detailed risk/benefit-assessment for the procedure, taking into account individual properties of the specific API[22]. The Biowaiver Monographs thus serve as aid in regulatory decision-making (including WHO's own Prequalification Committee) as to whether or not generic approval via BCS-based biowaiver can be justified for individual APIs and drug products thereof.

The BCS-based biowaiver is a promising tool enabling cost savings via comparative assessment of dissolution performance and reduction of the regulatory burden in the course of regulatory approval of generic drug products, and can thus help to facilitate the accessibility of essential medicines. There are, however, issues that prevent facile application of the procedure: ambiguities in solubility and permeability classification of APIs[23], drug products failing to meet regulatory dissolution criteria although being otherwise eligible[24], scientific doubts regarding the discriminatory power of the regulatory specifications to discern differences in dissolution performance relevant to the behaviour *in vivo* on the one hand[25–28] and suggestions for extending the procedure to certain poorly soluble drugs[29–34] along with arguments in favour of widening the regulatory specifications[35–39] on the other hand.

This dissertation aims to address these issues by providing a thorough assessment of the applicability and limitations of the BCS-based biowaiver in its current state. Potential modifications to the procedure are investigated and evaluated on the basis of experimental *in vitro* data and linked to the *in vivo* situation using *in silico* modelling and simulation tools.

Two subprojects presented in this thesis, namely establishing reliable BCS classifications for medicines recently added to the EML (PUBL. 1) and the preparation of a Biowaiver Monograph for folic acid (PUBL. 2), are closely linked to the aforementioned WHO and FIP initiatives and were funded in part by the WHO, which granted monetary resources to the WHO Collaborating Centre for Research on Bioequivalence Testing of Medicines at the Goethe University, Frankfurt am Main, Germany, for purchase of essential APIs.

1.2. Structure of the Thesis

This dissertation is prepared in a publication-based approach. It provides a comprehensive summary and discussion of the main results set out in the individual, peer-reviewed publications, which can be found in the Appendix. The scientific framework provides fundamental information covering all aspects relevant to the biopharmaceutical and regulatory assessment of the *in vitro* and *in vivo* performance of generic immediate release solid oral dosage forms and, more specifically, to the BCS-based biowaiver. Improvement of the data quality as well as an assessment of the current state of the procedure, its applicability to essential medicinal drug products and the investigation of possible extensions to the regulatory specifications are the key aims of the thesis. Results of the individual subprojects are discussed in the framework of current scientific developments. Based on these results, an outlook on potential future developments and experimental considerations is provided.

The published, peer-reviewed articles reprinted in the appendix to this dissertation are indicated in this thesis as “(PUBL. 1-6)”, where appropriate.

2. Scientific Framework

2.1. Biopharmaceutical Aspects of Drug Release

The biopharmaceutical and pharmacokinetic lifecycle of an API may be described on the basis of the LADME-Scheme[40], which divides the overall process into individual aspects: **L**iberation, **A**bsorption, **D**istribution, **M**etabolism and **E**xcretion. While distribution, metabolism and excretion are part of the post-absorptive pharmacokinetics and are usually unaffected by the choice and performance of an oral dosage form, liberation and absorption are linked more closely to the biopharmaceutical behaviour.

In order to characterize the environment in which liberation and absorption take place for APIs formulated in immediate release solid oral dosage forms, a brief overview of the physiology of the fasted upper gastrointestinal tract (GIT) is given.

2.1.1. Physiology of the Fasted Upper Gastrointestinal Tract

Pharmaceutical immediate release solid oral dosage forms are designed to rapidly disintegrate and release their active pharmaceutical ingredient(s) after contact with water or physiological digestive fluids. After oral administration with a glass of water, the dosage form passes through the esophagus, reaching the stomach and subsequently the small intestinal segments duodenum, jejunum and, potentially, the ileum. A schematic overview of the upper GIT is depicted in FIG. 1.

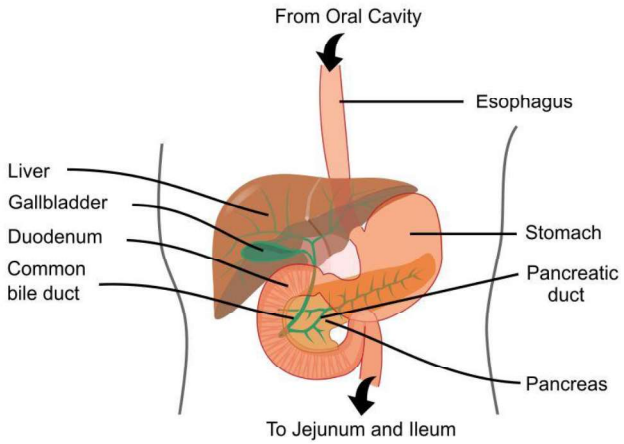

FIGURE 1: *Schematic anatomical structure of the upper gastrointestinal tract.*

The total volume of the gastric lumen is around 150-170 mL in the fasted state[41] with a residual fluid volume of approximately 25-40 mL[42,43], consisting mostly of swallowed saliva and fluid secretions from gastric mucosal cells, such that the gastric fluids are mainly composed of hydrochloric acid, sodium chloride, digestive enzymes such as pepsin and gastric lipase and, in some individuals, low amounts of bile salts from duodenal reflux[44]. The pH of the fluid is

acidic, with reported median values of 1.5 – 1.9 for the residual gastric fluid[45]. After intake of a glass (~200 mL) of water the pH can increase to a to a mean value of 2.7 (range of individual medians: 1.4 – 4.6) due to dilution effects, but then usually reverts to the basal pH over the course of several minutes due to luminal acid secretion from parietal cells[46]. Similarly, the physiological fluid temperature of 36-37°C briefly drops down to around 23°C after intake of a glass of water at room temperature, and reverts to the initial temperature within 10 minutes[46].

In order for drugs dissolved in physiological fluids to be absorbed, they first have to be emptied from the stomach into the small intestine, as the gastric mucosa is not suited to quantitative absorption due to narrow tight junctions, the absence of active transporters that could facilitate the transcellular apical to basolateral uptake and due to the relatively small (~600 cm²) total effective surface area available for transcellular absorption via passive diffusion[47], even when accounting for a slight amplification due to rugal folds[48,49].

After a brief and variable lag-time of up to 15 minutes, emptying of administered non-caloric fluid contents occurs relatively quickly and, depending on the total fluid volume, usually follows a first-order emptying kinetic with a half-emptying time of approximately 22 minutes (for 50 mL) or 12 minutes (for 200 mL), respectively[50]. In contrast, solid contents and particles larger than ~2 mm cannot pass through the pylorus and are emptied instead via *housekeeping waves*, which are peristaltic contractions of high intensity that occur in regular time intervals in the fasted state[49]. The gastric residence time in the fasted state is largely dependent on the contraction phase which is in turn determined by the migrating myoelectric complex (MMC)[49]. A distinction into three MMC phases is usually made in the literature, with each phase varying in duration and in the frequency of gastric contractions[50]. The MMC phases are the main reason for the high variability in gastric residence time of solid contents observed among human subjects in the fasted state, with a reported range of 4 – 233 minutes (median: 30 minutes)[46]. Hence, the MMC phase present during oral administration of solid dosage forms determines the average time until the next housekeeping wave occurs, culminating in the transfer of solid contents into the small intestinal lumen.

After being emptied from the stomach, contents enter the small intestine, which is the major site of absorption for most orally administered drugs. Anatomically, it is divided into the duodenum, jejunum and ileum. From proximal to distal sections, the pore diameter of epithelial tight junctions decreases. The potential contact surface area for a drug solution in the small intestinal lumen is increased largely due to structures such as Kerckring's valves, villi and microvilli, amounting to an effective area of 200 m² which facilitates passive absorption. In addition to the larger surface area, the enterocytes in the small intestine are specialized cells for absorption of nutritional components and express transporters on their apical cell membrane that facilitate the absorption of certain nutritional components (amino acids, di- and

tripeptides, mono-, di- and triglycerides)[49], and can also play a role in the absorption of certain drugs (e.g. amoxicillin and cephalosporins via hPEPT[51], levodopa via neutral or dibasic amino acid antiporters[52]).

The average small intestinal fluid content was reported as 46 mL in the fasted state and rises up to a mean 94 mL after oral administration of 240 mL of water, with a large variability and fluctuation due to water absorption and secretion. Further, the fluids were observed to not form one large coherent volume, but rather showed distribution into 'water pockets' (on average 15 individual fluid pockets with a volume of ~6 mL) in a study using magnetic resonance imaging techniques[42]. The median pH of the fluids in the small intestinal compartments is close to neutral (duodenum: ~6.3[53]; jejunum: ~6.9[53]; ileum: ~7.7[46]) and, as reported in studies analysing aspirates or using *in situ* pH measurement via telemetric devices such as the Intellicap® system, exhibits lower inter-subject variability and smaller intra-subject fluctuations compared to the gastric pH[46]. Mainly responsible for the change in pH of fluids that are emptied from the stomach and transferred to the duodenum are bicarbonate ions contained in secretions of the small intestinal mucosa and the pancreas, which are able to neutralize the acidic gastric fluids. In addition to the small intestinal secretion of electrolytes and bicarbonates and the pancreatic secretion of digestive enzymes (e.g. lipase, trypsinogen), the molecular composition of intestinal fluids is further determined by the presence and quantity of bile fluids[49]. Both, pancreatic secretions and bile fluids enter the duodenal lumen collectively via the major duodenal papilla that is situated in the descending part of the duodenum[49]. Bile fluids are alkaline (pH 7.5 - 8.05[54]) and consist of bile salts, bilirubin and lipid components such as cholesterol and lecithin, which improve the wettability of lipophilic substances and aid their solubilisation via micelle formation, thus facilitating their dissolution and absorption[49].

The specific biopharmaceutical behaviour of APIs formulated as IR SODF in the GI environment in the fasted state is primarily dependent on the inherent properties of the API. Most importantly, solubility and intestinal permeability of a drug molecule are key indicators of an API's oral bioavailability (BA).

2.1.2. Physicochemical and Biopharmaceutical Properties of the API

2.1.2.1. Solubility

The equilibrium solubility of an API in gastrointestinal fluids is the major factor limiting the total drug amount available for transcellular absorption and is thus one of the most important biopharmaceutical aspects. Per IUPAC definition[55], the equilibrium solubility of a substance (solute) in a certain medium (solvent) is the concentration of a stable saturated solution that forms over a bulk of solid, undissolved residue of the solute, coinciding with the chemical potential of the undissolved solute being equal to the chemical potential of the substance in

solution. Apart from interactions between the API and the solvent that can be estimated based on their individual physicochemical properties such as the dielectric constant of the solvent or the octanol-water distribution coefficient of a solute[56], the solubility of an API can be influenced by external circumstances such as the temperature of the solvent but also by other factors such as the simultaneous presence of other dissolved substances. Common-ion and diverse-ion effects can play a role in the solubility of salt-forming APIs[57], and solubilisation via physiological bile salt micelles[58] or mediated through excipients such as cyclodextrins[59] that may be present in a dosage form have to be taken into account when evaluating the solubility of an API under physiologically relevant conditions.

Another major factor influencing the solubility of an API with acidic or basic functional groups is the pH of the solvent. According to the *Henderson-Hasselbalch equation*[60,61], the solubility of ionized species changes logarithmically with changing pH. Based on the melting point, logarithmic octanol-water distribution coefficient (LogP) and acid (pK_a) or base (pK_b) dissociation constants of an API, the pH-solubility profile may be estimated by combining the *general solubility equation* (GSE)[62] and the Henderson-Hasselbalch equation. As the physiological pH in the GIT ranges from acidic in the stomach to slightly basic in the ileum, the solubility between pH 1 and 7 is of special interest when assessing the potential *in vivo* behaviour of a drug. More importantly, as gastric emptying is associated with a sudden rise in pH due to the physiological environment in the duodenum, some drugs with basic functional groups may either supersaturate or precipitate during the transition from the stomach to the intestines[63]. Thus, the amount of drug eventually available for absorption can be influenced largely by the pH-dependent solubility of ionisable compounds.

2.1.2.2. Intestinal Permeability

After liberation from a pharmaceutical dosage form and dissolution in the GIT, solubilised drug molecules may be absorbed from the small intestinal lumen by a variety of pathways[49,64]: passive transcellular diffusion, active or facilitated transcellular uptake, or paracellular diffusion (for very hydrophilic drugs). Endocytosis of undissolved particles via microfold cells of the Peyer's patches is considered negligible[65]. For the majority of APIs, passive transcellular diffusion is the predominant absorption pathway[66] and therefore determination of an API's permeability across the membrane of enterocytes is of great interest for predicting the rate and extent of the absorption process. Permeability can be mathematically described with EQ. 1 as the rate of penetration of a substance into a membrane where ***D*** is the diffusion coefficient of the substance in the membrane, ***K*** is the partition coefficient between the membrane and the surrounding aqueous phase and ***L*** is the thickness of the membrane[67]:

$$Permeability = \frac{D_{Membrane} \cdot K_{\frac{Membrane}{Aqueous}}}{L_{Membrane}} \qquad \text{(EQ. 1)}$$

For determination of the intestinal permeability of an API, various approaches are available that differ in their predictive power and representation of the physiological situation, ranging from pure *in silico* estimation based on quantitative structure-activity relationships using molecular descriptors[68] through *in vitro* experiments using artificial membranes[64] (e.g. PAMPA) or cultured cell-monolayers (Caco-2, MDCK)[69] to *in vivo* perfusion studies in animals[70] or humans[71].

In silico predictions are most useful for early screening of potential new drug candidates as they are cheap and rapidly performed, but in most cases correlate only moderately with experimentally determined values[72].

The *in vitro* permeability assessment of a compound in studies using Caco-2 or MDCK cells is well established and widely used[72]. The apparent permeability value (P_{app}) in these studies is calculated from the steady-state substance flux (apical to basolateral) between two chambers separated by a cultured monolayer of human colonic adenocarcinoma cells (Caco-2) or Madin-Darby canine kidney epithelial cells (MDCK)[66]. Compared to studies that use simple artificial membranes, active transport can be additionally represented in MDCK cell lines, as they can be transfected to express certain metabolic enzymes or transporter proteins on their cell surface, allowing for a more mechanistic permeability assessment[72]. While good rank-order permeability correlations and correlations with effective intestinal permeability (P_{eff}) in humans or the total fraction absorbed (f_{abs}) *in vivo* can be achieved in Caco-2 assays, a major drawback of the method is the large inter-laboratory variability, as the measured permeability is very sensitive to the exact composition of the buffer media used in the assay as well as to the specific properties of the cultured cell line[72,66]. A more reliable source of permeability data but also more laborious to perform is the determination of the effective permeability of individual intestinal segments via *in situ* intestinal perfusion models using either a closed-loop[73] or a continuous perfusion[74] approach in animals and humans.

In addition to the above-mentioned approaches, which assess individual aspects and sub-processes of drug absorption, oral bioavailability (BA)[75] and mass-balance studies[76] in humans can also be used to assess the fraction (f_a) of drug absorbed from the GIT based on either the amount of drug found in the blood plasma (BA) or calculated from intact or metabolized, radio-labelled drug molecules recovered post-administration from excretion pathways (mass-balance). In combination with knowledge about a drug's solubility in the GIT, the permeability or the f_a of a drug can be used to assess potential limitations to the oral BA of a drug.

2.1.2.3. Concept of the Biopharmaceutics Classification System

In 1995, Amidon and co-workers[77] established the concept of a simple classification system for APIs based on two biopharmaceutical aspects, drug solubility and permeability, creating the *Biopharmaceutics Classification System* (BCS). The approach was adopted by the United States Food and Drug Administration (FDA) in 2000[78] as the first regulatory authority that applied the concept to the assessment of bioavailability problems of solid oral drug products. However, the term 'permeability' in this context is misleading, as the BCS takes the total fraction absorbed from the gut lumen into account, rather than a specific permeability value, by classifying drugs that are absorbed to an extent of at least 85% as *highly permeable*, as e.g. in the current, revised FDA guidance[4]. Regarding the solubility classification, a drug is assessed on the basis of its dose/solubility-ratio (DS) and is considered *highly soluble* when the complete dose of an API can be dissolved in 250 mL of buffered media (DS ≤ 250 mL) over the relevant physiological pH range (pH 1 – 6.8)[4]. Combination of both criteria results in the four BCS classes depicted in FIG. 2.

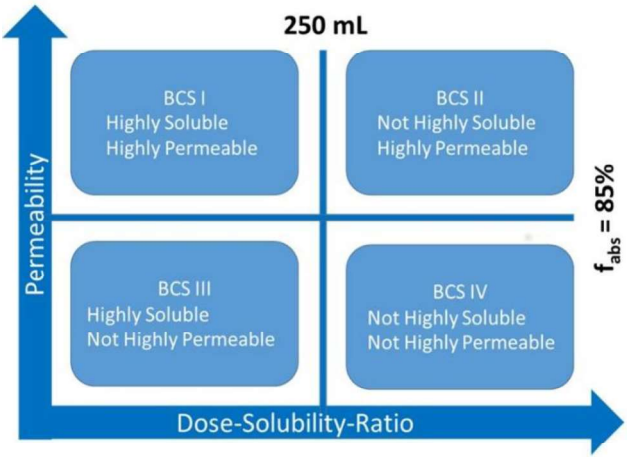

FIGURE 2: *Current FDA interpretation of the Biopharmaceutics Classification System[4] [reprinted from PUBL. 1 with permission from Elsevier].*

As solubility and permeability are considered to be the most crucial aspects for the assessment of the oral bioavailability of a drug and due to the simplicity of categorizing the entirety of drug molecules into four groups, the BCS classification has been and is widely used[23,79,80]. Additionally, its field of application has diversified from the originally intended regulatory use to other fields such as drug and formulation development[67,81,82]. Along with the standard BCS (FIG. 2), modifications have been proposed that either address the suitability of the classification criteria or tailored the whole classification system more towards goals other than the original regulatory applications. As an example, due to the difficult and laborious experimental assessment of the fraction absorbed and the resulting ambiguities in the permeability classification, Wu and Benet[83] proposed the replacement of the BCS permeability

criterion in the FDA interpretation in favour of the experimentally more easily accessible total extent of drug metabolism, resulting in the *Biopharmaceutics Drug Disposition Classification System* (BDDCS). For the industrial use in formulation development, Butler and Dressman[84] proposed a *Developability Classification System* (DCS) that was later refined by Rosenberger et al.[85] to further facilitate its practical implementation. With the aim of improving the BCS in terms of physiological relevance while maintaining its overall simplicity, several aspects were added in the DCS including consideration of the interplay between permeability and intestinal solubility, highlighting that a lower solubility may be compensated by rapid absorption *in vivo*. Furthermore, the DCS contemplates the importance of aspects tied to the performance of the dosage form, such as the dissolution rate and particle size distribution - aspects also discussed in the BCS, but unheeded in the classification of an API.

2.1.3. Biopharmaceutical Performance of Solid Oral Dosage Forms

In addition to the intestinal solubility and permeability of an API, its liberation from the pharmaceutical dosage form is a fundamental requirement for subsequent absorption and can be characterized by two processes: disintegration of the pharmaceutical IR SODF and dissolution of individual drug particles[86]. The behaviour of a specific dosage form is highly dependent on the interplay between the physicochemical characteristics of the API, the process parameters applied during manufacture and the excipients used in formulation development.

In the context of this thesis, the focus was dedicated to excipients and aspects related to disintegration and dissolution of tablets, rather than capsules. Thus, all drug products subjected to dissolution and disintegration experiments were tablet formulations.

2.1.3.1. Excipients and their Influence on the Biopharmaceutical Behaviour

Pharmaceutical formulations for oral administration must ensure dosing accuracy and API stability, facilitate the handling for the patient and, desirably, be aesthetically appealing. When no modification of the drug's biopharmaceutical behaviour or its pharmacokinetics is required to ensure therapeutic efficacy, IR SODF such as tablets and capsules are the formulation of choice as they comply with the aforementioned requirements, can be manufactured with high-throughput machinery and are generally well received by patients and caregivers[87,88]. To facilitate manufacturing of the dosage form and easy handling by the patient, excipients are usually added to the API prior to the tablet compression or encapsulation step.

Excipients are added to the API for various reasons: inert filler materials to enhance the bulk volume; binders to increase the mechanical stability of the resulting dosage form; and flow regulating agents, glidants and mold-release agents to guarantee smooth operation of the tableting process and prevent sticking, segregation and other dosage inaccuracies[89,90]. While

these excipients facilitate high-throughput tableting, glidants and mold-release agents such as magnesium stearate can negatively influence the wettability of the tablet surface, as these excipients are lipophilic and can thus retard water penetration into the tablet core[91,92]. In order to improve release of the API from the dosage form after contact with an aqueous medium, surface active agents (surfactants) that improve tablet wetting or excipients facilitating physical disintegration (disintegrants) may be added[89]. Many potent disintegrants (e.g. croscarmellose sodium or sodium starch glycolate) accelerate the disintegration process via swelling, as they consist of cross-linked hydrophilic polymers that are insoluble in water but highly hygroscopic and swellable[93]. Other modes of action of disintegrants facilitating the disintegration of a tablet include wicking of water (e.g. starch) or *in situ* release carbon dioxide via reaction of bicarbonates and organic acids in effervescent tablets after contact with an aqueous medium[86].

Other excipients such as flavouring agents or colorants may be added during manufacture of a SODF for either aesthetic or patient compliance purposes. However, they usually have no or only negligible influence on the product's biopharmaceutical behaviour[4].

Potential excipient effects are not limited to directly affecting the disintegration process. They may also (in most cases unintended) influence the biopharmaceutical behaviour via modification of the solubility and dissolution rate[94], membrane permeability[95,96] or small intestinal transit time[97,98] of a drug. The excipients that can potentially influence the bioavailability of an API are often referred to as 'critical excipients'. Well documented examples of such interactions caused by critical excipients reported in the pharmaceutical literature are sodium lauryl sulphate (SLS), Macrogol 400 or hydroxypropyl methylcellulose (HPMC)[99,100,95]. However, as the excipient concentrations studied in the reported examples often vastly exceed amounts usually used in IR SODF, and as some excipient effects are specifically linked to certain APIs, their influence on the biopharmaceutical behaviour cannot be generalized to all SODF. Instead, it is important to consider the quantitative as well as qualitative excipient composition of each product to determine whether the excipients are likely to affect the API bioavailability.

2.1.3.2. Disintegration of Tablet Formulations

While formulating an API as a tablet facilitates its overall handling and oral administration, an additional biopharmaceutical hurdle is introduced as the API needs to be released from the dosage form via disintegration of the tablet core in order to become more available for dissolution and subsequent absorption.

The disintegration of a tablet in an aqueous medium is a complex physical procedure. The exact process depends on individual factors such as the composition of the fluid medium, the

type and quantity of excipients as well as the parameters used in the manufacturing process. Fundamental aspects in the theoretical description of disintegration are wetting of the tablet surface area followed by liquid penetration that can lead to swelling, strain recovery or dissolution of individual particles. All three mechanisms eventually result in the interruption of inter-particular bonds such as solid bridges, mechanical interlocking or intermolecular bonds that were either introduced or reinforced during the manufacturing process.[86]

The mechanistic investigation of dosage form disintegration and its separation from dissolution of individual particles is challenging, as both processes usually occur simultaneously. Experimental approaches therefore focus on quantifying the early disintegration processes that occur when a dosage form comes into contact with a fluid: liquid permeation into pores and the subsequent swelling of individual particles or the tablet matrix as a whole[86,101].

Characterizing aspects of the disintegration process *in vitro*, the overall pore volume of a tablet can be determined using compendial porosimetry techniques that assess the pressure dependent permeation of gases (e.g. helium[102]) or liquids (e.g. mercury[103]) into the dosage form. In addition, imaging techniques such as x-ray microtomography can be used to gain insight on the spatial distribution of pore sizes[104,105]. Liquid penetration into pores and the subsequent swelling of the tablet or individual particles may be assessed using magnetic resonance imaging (MRI) and terahertz time-domain spectroscopy (THz-TDS) or terahertz pulsed imaging (TPI)[86,106,107].

The *in vivo* disintegration behaviour can be assessed using tracer techniques such gamma scintigraphy, where a radiolabelled substance is incorporated in the dosage form[108,109], or with the help of magnetic resonance imaging using incorporated magnetic substances such as ferrous oxide for visualization of dispersion of particles in the course of disintegration[107].

Due to the experimental challenges associated with simultaneously assessing the numerous underlying mechanisms, disintegration is often described empirically in pharmaceutical science. Approaches for quantitative empirical description of the disintegration process range from a simple visual determination of the time t_{Disint} required for complete disintegration of a pharmaceutical dosage form[101][110], through numerical calculation of a theoretical disintegration profile[111,112], to a more mechanistic assessment based on mathematical modelling of the processes considered fundamental to disintegration.[113,114]

2.1.3.3. Dissolution of Drug Particles

The dissolution rate of individual particles can be mathematically described with an equation that was first postulated by Noyes and Whitney[115] and later expanded by Nernst[116] and Brunner[117] to the *Noyes-Whitney/Nernst-Brunner equation*:

$$\frac{dC(t)}{dt} = \frac{D \cdot A(t)}{V \cdot h} \cdot [C_s - C(t)] \qquad \text{(EQ. 2)}$$

According to this equation, the change in concentration ***C(t)*** over the time interval **d*t*** of a substance dissolving in a specified medium is dependent on the diffusion coefficient ***D*** of the substance in the medium, the medium volume ***V***, the total surface area ***A(t)*** of undissolved particles, the thickness of a hydrodynamic diffusion layer ***h*** surrounding the undissolved particles and the concentration gradient between the solubility $\boldsymbol{C_s}$ of the drug at equilibrium and the momentary drug concentration at time **t**, ***C(t)***.

Many variations of this equation exist and describe the dissolution process at different levels of complexity. One version is the *z-factor dissolution model*[118,119], which assumes the drug particles to be nonporous, spherical and uniform in size. This allows the particle mass to be used instead of the particle surface area under consideration of the particle density ***ρ*** and the surface to volume ratio of a sphere ($\frac{\mathbf{3}}{\boldsymbol{r}}$). Transposition of EQ. 2 under these assumptions leads to the following equation:

$$-\frac{dM_s(t)}{dt} = \frac{3D}{h \cdot \rho \cdot r_0} \cdot M_0^{\frac{1}{3}} \cdot M_s(t)^{\frac{2}{3}} \cdot [C_s - C(t)] \qquad \text{(EQ. 3)}$$

where $\boldsymbol{M_0}$ is the total initial particle mass available for dissolution, $\boldsymbol{M_s(t)}$ is the total undissolved particle mass at time ***t*** and $\boldsymbol{r_0}$ is the initial particle radius. Assuming that $\frac{\boldsymbol{D}}{\boldsymbol{h}}$ remains constant during the dissolution process, the term $\frac{\boldsymbol{3D}}{\boldsymbol{h \cdot \rho \cdot r_0}}$ can be replaced by a hybrid dissolution factor ***z***:

$$-\frac{dM_s(t)}{dt} = z \cdot M_0^{\frac{1}{3}} \cdot M_s(t)^{\frac{2}{3}} \cdot [C_s - C(t)] \qquad \text{(EQ. 4)}$$

Other approaches based on EQ. 2 are the *Johnson dissolution model*[120], which describes the dissolution of spherical particles of different initial sizes, or the *Wang-Flanagan dissolution model*[121,122], which further accounts for non-spherical particles by applying an individualized shape-factor.

All these dissolution models can be used to describe an observed *in vitro* dissolution behaviour of a pharmaceutical dosage form and may subsequently be used to simulate the *in vivo* performance by using the parameterized data as an input variable in biopharmaceutical *in silico* modelling software.

2.2. Experimental Considerations for *in vitro* Drug Release Testing

Investigating the API release from a pharmaceutical dosage form requires a thorough prior assessment of the biopharmaceutical properties of an API (e.g. BCS class I or IV?), its dosage form characteristics (e.g. immediate or modified release formulation?) and the scientific problem to be investigated (e.g. comparative QC testing or prediction of the *in vivo* performance?) in order to find a suitable experimental setup that keeps the balance between sufficient model complexity and cost- and time-effective experimental feasibility. Providing detailed guidance for the choice of suitable experimental setups, results from the EU research initiative *OrBiTo* have been recently compiled in publicly available decision trees[123].

In the following sections, the particularities and intended purposes of the most prevalent instrumental setups and dissolution media used for dissolution testing of IR SODF are presented.

2.2.1. Choice of Dissolution Apparatus Setup

The first instrumental setups for dissolution testing that were adopted by regulatory authorities in the 1970s were the United States Pharmacopoeia (USP) apparatus I (*basket apparatus*) and II (*paddle apparatus*)[124]. According to the current Pharmacopoeia of the US[125] and the EU[126], they consist of an assembly with 6-12 individual positions for dissolution testing. Each position can hold a cylindrical glass vessel with a capacity of up to 1 L dissolution medium. To maintain a physiologically relevant temperature of 37 ± 1°C, the vessels are heated by a surrounding water bath. In the USP apparatus I, the dosage form to be tested is placed inside a cylindrical basket comprising a metal mesh that is rotated around its vertical axis with a constant speed, usually 100 revolutions per minute (RPM). In contrast, when operating the USP apparatus II, the dosage form is placed directly into the dissolution medium and usually sinks to the lowest point in the hemispherical vessel. The contents of the vessel are stirred with a stainless steel paddle that is immersed in the dissolution medium, usually applying 50 to 75 RPM. After placing the dosage form in the dissolution medium, aliquots are withdrawn at specified time intervals and their API content is quantified with a suitable analytical method such as high performance liquid chromatography (HPLC) or UV/VIS spectroscopy. The typical setup of a dissolution test using USP apparatus I and II is schematically depicted in FIG. 3.

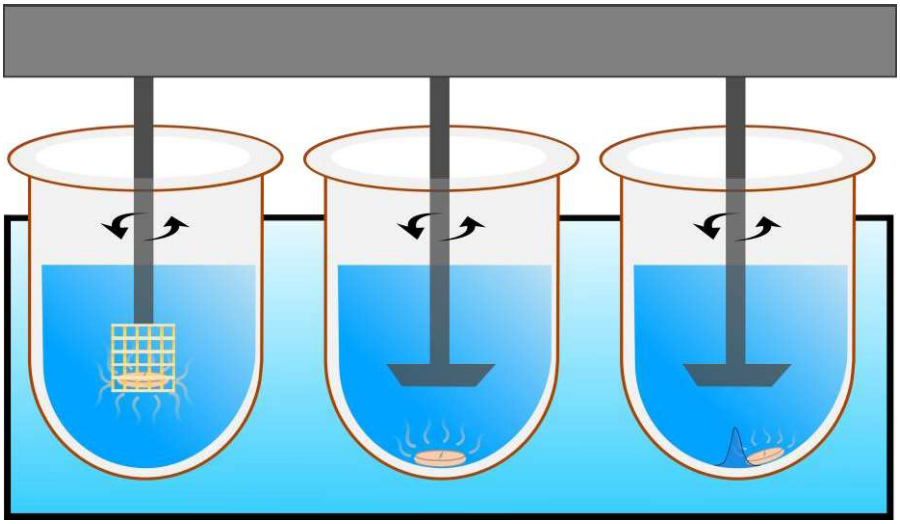

FIGURE 3: *Release testing of a tablet formulation in different dissolution test setups.* ***From left to right:*** *USP apparatus I, USP apparatus II, USP apparatus II using Peak Vessels™.*

The primary intended purpose of the compendial dissolution test setup is the assessment of uniformity of dosage form performance under the aspect of QC testing and batch release in the pharmaceutical industry. However, due to the standardization of the apparatus and their ubiquitous laboratory use, the compendial setup is also frequently adopted for predicting the potential *in vivo* behaviour of a dosage form. Aiming to resolve typical problems encountered during compendial dissolution testing or to improve the physiological relevance, several modifications to the instrumental setup have been proposed[127]. Different vessel types were developed that can, in the case of *Peak Vessels™* (FIG. 3), reduce the occurrence of the dosage form becoming trapped in a zone with low hydrodynamic movement (usually referred to as 'coning') when using the USP II apparatus[128,129], or allow the use of smaller media volumes (e.g. *Mini Vessels*, FIG. 4) to better represent physiological fluid volumes. Changes in the experimental procedure include two-stage testing[130] or application of the transfer-model[63]. Both procedures aim to simulate the transition of the drug product from the gastric into the small intestinal environment by either abruptly changing the media composition mid-test (two-stage testing) or by continuously transferring volume aliquots from one vessel simulating the gastric environment into a second vessel simulating the intestinal environment (Transfer Model, FIG. 4).

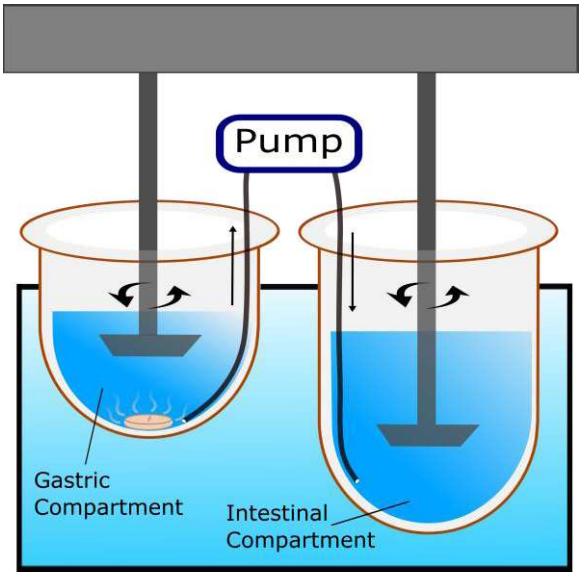

FIGURE 4: *Schematic setup of the Transfer Model developed by Kostewicz and co-workers[63]. The contents of a Mini-Vessel representing the gastric environment are transferred into a second, regular vessel representing the intestinal environment.*

Apart from the instrumental setups that are based on the regulatory dissolution testers, other experimental approaches exist that primarily aim to closely mimic the conditions present in the GIT. Absorptive processes in the small intestine happening concurrently with luminal drug dissolution can be simulated by bringing the dissolution medium in contact with an organic phase[131], an artificial membrane[132] or a cultured cell monolayer[133] as the absorptive compartment. Realistic temperature and pH profiles in the fasted stomach as well as pressure events during gastric emptying can be simulated in the *GastroDuo* apparatus[134]. Attempting to simulate the complete GIT passage, the TNO (Gastro-)Intestinal Model (TIM) is able to simulate the transit of a dosage form through an array of compartments, mimicking many physiologically relevant aspects such as peristaltic contractions, secretion of digestive fluids, absorption processes and prandial states[135].

2.2.2. Choice of Suitable Dissolution Media

In addition to the variety of instrumental setups available for release testing, many different dissolution media can be used for dissolution testing.

Similar to the choice of the instrumental setup, the suitability of a medium for release testing of solid oral dosage forms depends on the biopharmaceutical aspects of the API (e.g. its BCS classification), the complexity of the dosage form and the expected interactions in the gastrointestinal environment. Addressing this issue and providing guidance for media selection, Markopoulos et al.[136] summarized the key aspects to be considered in specific experimental scenarios and created a corresponding rank-order system for dissolution media based on their level of complexity, ranging from Level 0 to 3.

Ideally, the dissolution medium should reflect the physiological environment as closely as possible in regard to its qualitative and quantitative media composition and properties that may affect dissolution such as bulk pH, buffer capacity, osmolality and surface tension. However, a reasonable balance between media complexity, chemical costs and simplicity of media handling and sample analysis is usually sought in experimental practice, as dissolution tests become more laborious with increasing media complexity and high levels of physiological relevance are not always needed for an adequate representation of a drug's potential *in vivo* behaviour.

For highly soluble, ionisable compounds in IR dosage forms, dissolution testing in media that only account for a physiologically relevant pH (*Level 0 media*[136]), for example compendial Simulated Gastric Fluid (SGF), can be considered appropriate. For these compounds, the pH is the main aspect determining their solubility and thus their dissolution rate. However, in many cases, the buffer capacity of the medium needs to be taken into account in addition to the bulk pH (*Level 1 media*[136]). Mimicking the physiological conditions with regard to buffer capacity is complicated by two factors: first, the buffer capacity in fasted state physiological fluids originates mainly from dissolved bicarbonates, which are unstable due to potential conversion to carbonic acid and subsequent decomposition to carbon dioxide, and is thus highly dependent on the equilibrium between the individual components[137–139]. Although instrumental setups have been developed that enable use of media with a bicarbonate buffer system (e.g. *pHysio-grad*®, https://physiolution.eu/), other, more stable buffer systems (such as phosphate, maleate or acetate buffers) are usually chosen for practicability and economic reasons. Second, the measured buffer capacity in physiological fluids is comparably low[140], as the pH in the GIT is mainly affected by either secretion of hydrochloric acid (in the stomach) or intestinal secretions, bile and pancreatic fluids (in the small intestine) rather than being controlled by the total amount of dissolved buffer components[141,142]. This further hinders an exact *in vitro* representation, as low buffer capacity is a source of variability and hence poor reproducibility of experimental results. Therefore, most compendial dissolution media such as Simulated Intestinal Fluid (SIF) exhibit a higher buffer capacity (TBL. 1), foregoing an exact physiological representation in favour of experimental feasibility. In addition to stabilizing the bulk pH of a dissolution medium, the molar concentration of buffer components can also affect the pH in the microenvironment surrounding individual drug particles and can therefore be a key aspect for appropriately simulating *in vivo* conditions in some scenarios, as was recently demonstrated for the dissolution behaviour of ibuprofen[33,143].

Based on scientific advances in the characterization of the human GIT and the inability of compendial dissolution media to adequately reflect the *in vivo* behaviour of certain APIs, especially those with poor aqueous solubility, *biorelevant media* were developed in the late 1990s in order to improve the predictive power of *in vitro* dissolution testing[144]. In addition to a physiologically relevant bulk pH value and buffer capacity, they include components such as bile salts and lipids in physiological quantities and maintain a realistic osmolality (*Level 2 media*[136]). The inclusion of bile salts and lipids enables the formation of micellar structures in the dissolution medium, which can facilitate the solubilisation of lipophilic compounds. Further, the amphiphilic bile salts reduce the medium's surface tension and can thus improve wetting of particles and liquid permeation into porous structures, both of which are key elements in the disintegration process of solid oral dosage forms. Fasted State Simulating Gastric Fluid[45] (FaSSGF) and Intestinal Fluid[144] (FaSSIF) were the first biorelevant media that were developed. The latter of which has been modified two times[145,146] based on new insights into the composition of intestinal fluids, resulting in the latest version *FaSSIF-V3* which closely resembles human intestinal fluid in its qualitative and quantitative composition as well as in measurable physicochemical properties (TBL. 1). Following the development of media simulating the fasted prandial state, fed state media[144,145] (FeSSGF and FeSSIF) were designed. These reflect the composition of gastric and intestinal fluids after the intake of a standardized meal such as the standardized FDA breakfast administered in pharmacokinetic studies investigating the effects of food on the bioavailability of an API.

Media discussed so far that range from *Level 0* to *2* in regards to their complexity are adequate for simulating the *in vivo* dissolution behaviour of most drugs formulated as IR SODF. For highly soluble drugs (BCS classes I/III), compendial media can provide sufficient physiological relevance albeit their simple composition, while *Level 2* media such as FaSSGF and FaSSIF are the preferred choice for dissolution testing of BCS class II/IV drugs[136].

The use of *Level 3* media, as described by Markopoulos et al.[136], will seldom be a necessity for IR SODF, as these media further include digestive enzymes, dietary proteins and simulate viscosity effects that are considered important for specific lipid-based formulations, modified release dosage forms or the simulation of interactions in the fed state that cannot be adequately assessed with *Level 2* media.

The composition of key media used for dissolution testing of IR SODF and their physiological counterparts are summarized in TBL. 1.

TABLE 1: *Properties and qualitative composition of selected dissolution media used for dissolution testing of immediate release dosage forms in comparison to physiological fluids in the fasted state.*

	$SGF_{(sp)}$ [147]	$SIF_{(sp)}$ [147]	FaSSGF[45]	FaSSIF (Original Version)[144]	FaSSIF (Version 3) [146]	Human Gastric Aspirates	Human Intestinal Fluid
Level of Complexity[136]	Compendial Media, Levels 0 – I		Biorelevant Media, Level II			Physiological fluids	
Qualitative Composition	NaCl HCl (Pepsin) Water	NaCl NaOH KH_2PO_4 (Pancreatin) Water	NaCl HCl Taurocholate Lecithin Water	NaCl NaOH KH_2PO_4 Taurocholate Lecithin Water	NaCl NaOH KH_2PO_4 Taurocholate Lecithin Glycocholate Lysolecithin Sodium oleate Cholesterol Water	NaCl KCl HCl Bicarbonates Bile Salts Pepsin Water	NaCl KCl Bicarbonates Bile Salts Digestive Enzymes Lipids Phospholipids Water
pH	1.2	6.8	1.6 or 2.0	6.5	6.7	1 - 3[45,43]	4.8 – 8.2[141]
Buffer Capacity [mmol/L/ΔpH]	N/A	18.4[148]	N/A	12[149]	5.6	7 (after water ingestion)[43]	2.3 – 13[150,146]
Osmolality [mOsm/kg]	180-200[151,152]	113[148]	121	270	215	98-140[43]	124 – 266[150]
Surface Tension [mN/m]	Similar to distilled water (68-72)[153]		42.6	54.7[149]	35.1	35 – 47[45]	28 - 46[150,146]

2.3. Linking API Properties and *in vitro* Drug Release to *in vivo* Pharmacokinetics

Evaluation of oral bioavailability and the potential impact of differences in the dissolution behaviour observed among dosage forms *in vitro* and subsequent prediction of the pharmacokinetics *in vivo* requires theoretical models and approaches that are able to empirically or mechanistically correlate *in silico* and/or *in vitro* with *in vivo* scenarios. In the following, a brief overview of established qualitative and quantitative approaches is given.

2.3.1. Qualitative and Semi-Quantitative Estimation of Oral Bioavailability

A simple, qualitative approach to assessing the oral bioavailability of an API based on molecular descriptors are the *Lipinski's rules of five*[154,155]. Correlating calculable molecular parameters to absorption and permeability, Lipinski and co-workers postulated that poor oral absorption is expected when two or more of the following characteristics apply: molecular weight > 500 Da, (calculated) Log P > 5, > 5 hydrogen bond donors, > 10 hydrogen bond acceptors. This postulated rule is based on the fact that less than 10% of orally administered compounds that enter clinical phase II trials exhibit a combination of two or more of the aforementioned calculated properties.

While enabling a quick estimation of the oral bioavailability, the *rules of five* do not account for the dynamic interaction between the drug and the small intestinal environment. A simplistic, mathematical description of the process of particles transiting the small intestine is the plug-flow model[77,156], which provides a semi-quantitative assessment of oral bioavailability on the basis of biopharmaceutical properties. In this model, the small intestine is represented as a cylindrical tube with defined length ***L*** and radius ***R*** through which a disc-like plug of fluid volume ***V*** containing (un)dissolved drug particles traverses at a constant flow rate ***Q*** (FIG. 5). Drug particles are assumed to dissolve according to the *Noyes-Whitney/Nernst-Brunner equation* (EQ. 2), and may permeate through the wall of the cylindrical tube on the basis of a constant, defined permeability value.

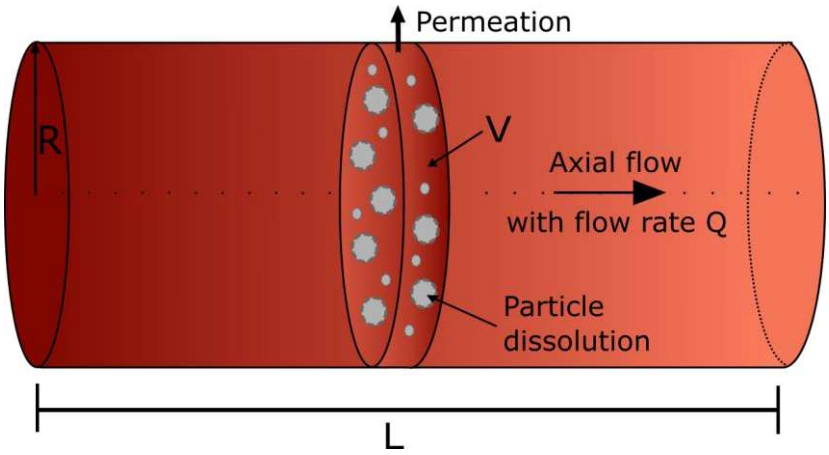

FIGURE 5: *Schematic depiction of the Plug-Flow-Model.*

Based on the plug-flow model, dimensionless parameters can be calculated from bio-pharmaceutical properties of an API to semi-quantitatively assess the interplay between solubility, dissolution and absorption: the dose number D_0, absorption number A_n and dissolution number D_n[77,157]. D_0 represents the multiple of a certain volume (usually 250 mL, as this approximately represents the volume of a glass of water in addition to the residual volume of the fasted stomach) needed to completely dissolve a given API dose. A_n represents the ratio between the time needed for complete absorption and the total transit time of the fluid plug through the cylindrical tube (estimated as 3 h in the BCS[77] and 3.32 h in the rDCS on the basis of *in vivo* small intestinal transit times[85]). D_n is the ratio between the total transit time and the time needed for complete dissolution of an individual drug particle. The relationship between dose, absorption and dissolution number of an API was of fundamental importance in the establishment of the BCS[77] and is also used in the rDCS[85] to semi-quantitatively assess the developability of drug candidates. The BCS or rDCS classification provides information about the biopharmaceutical properties limiting oral bioavailability: class I: no limitation expected; class II: solubility and/or dissolution; class III: permeability and/or absorption; class IV: solubility in addition to permeability/absorption.

All of the aforementioned approaches are applicable to the qualitative or semi-quantitative prediction of oral bioavailability based on *in silico* and *in vitro* data for the assessment of factors crucial for oral bioavailability. When supportive *in vivo* data are available, quantitative correlations between *in vivo* absorption and *in vitro* release testing may also be established.

2.3.2. Quantitative *in vitro in vivo* Correlation (IVIVC)

Establishing an IVIVC serves as validation tool for demonstrating the suitability of experimental *in vitro* conditions to reflect the physiological aspects relevant for drug release and thus enables subsequent quantitative prediction of the extent of absorption *in vivo*[158].

An IVIVC can be established via deconvolution of *in vivo* plasma concentration profiles using numerical or model-dependent approaches such as the Wagner-Nelson[159] (one compartment) or Loo-Riegelman[160] (two or three compartments) method to obtain an estimate of the *in vivo* absorption profile. The time course of the plasma concentration of an orally administered drug is mainly determined by two diametric processes, the appearance of drug in the systemic circulation as a result of intestinal absorption and its clearance as a result of distribution, metabolism or excretion. A mathematical description of the process in which a drug is cleared from the blood plasma and estimation of the amount cleared from an observed plasma concentration profile provides the basis for calculation of a theoretical *in vivo* absorption profile[161,162].

IVIVCs of sufficient quality are obtained only when *in vivo* absorption is assumed to be rapid in comparison to the dissolution rate, so that dissolution can be considered the major factor limiting oral bioavailability[161,162]. This is usually the case for sustained release dosage forms, but is only valid for certain specific IR dosage forms, depending on the formulated API and the dosage form characteristics. As the small intestine is the main site of absorption for the majority of drugs, gastric emptying time is a large confounding factor, especially for highly soluble (BCS I/III) or rapidly dissolving APIs, when trying to correlate the amount dissolved *in vitro* and the amount absorbed *in vivo*. Disintegration of an IR formulation is usually complete before gastric emptying, and dissolution of drug particles may therefore at least partly take place in the stomach, thus introducing a time-gap between dissolution and absorption defined by gastric emptying kinetics[163]. Depending on the BCS class of an API, the amount of drug reaching the systemic circulation can be solubility- or dissolution-limited (BCS II), permeability-limited (BCS III) or limited by all three aspects (BCS IV) and is rarely limited solely by dissolution (only for certain BCS I and II drugs). As a result it is usually difficult to establish an IVIVC for IR SODF[77].

For a reliable, quantitative estimation of the *in vivo* bioavailability of solid oral dosage forms, other approaches are therefore necessary to put the drug's dissolution behaviour into the context of the conditions present in the GIT, such as the dynamic transition between

physiological compartments with different biopharmaceutical characteristics (e.g. pH, fluid volume, bile salt concentration or intestinal surface area available for absorption). This can either be established by closely mimicking all physiological aspects in a single, complex experimental *in vitro* setup (e.g. GastroDuo[134] or TIM[135]) or by assessing and parameterising individual components of the biopharmaceutical behaviour separately, and then combining them with the help of sophisticated physiologically based *in silico* models[164].

2.3.3. Physiologically Based Biopharmaceutical Modelling (PBBM)

Physiologically based biopharmaceutical modelling has evolved from simpler models such as the plug-flow model describing intestinal drug transit and aims to provide an accurate, virtual representation of the entirety of biopharmaceutical and pharmacokinetic processes in the human body. While still dividing the human body into virtual compartments, as is also done in compartmental pharmacokinetics, the individual compartments in this case mimic physiological organs in their virtual size and function instead of being unspecified compartments solely defined by empirically fitted distribution rate constants[165].

A variety of commercial and open-source software with or without graphical interface exist that either allow the creation of user-defined models (e.g. Stella® Architect, Matlab®, Phoenix® WinNonLin®) or provide predefined model setups (e.g. SimCyp®, GastroPlus®, PK-SIM®)[164]. In the following discussion, the functions and general model setup of GastroPlus® is described in greater detail, as this software was used for the *in silico* simulations presented in this thesis.

The fundamental model setup of GastroPlus® is schematically illustrated in FIG. 6. Based on input parameters for biopharmaceutical properties of an API such as molecular weight, Log P, pH-solubility profile and P_{eff}, its dissolution, transition and absorption in a virtual gastrointestinal tract is simulated based on the *Advanced Compartmental and Transit* (ACAT) model[166]. The ACAT model divides the human GIT into 9 virtual compartments: *Stomach*, *Duodenum*, *Jejunum 1 - 2*, *Ileum 1 - 3*, *Caecum* and *Ascending Colon*. Each compartment is characterized by a set of parameters, amongst others defining its spatial dimensions, residual fluid volume and pH, transit time, effective surface area or fluid secretion and absorption rate. Further, a variety of options for customization are available, allowing the simulation of different prandial states, dosage forms, dissolution kinetics or fluid models.

The post-absorptive pharmacokinetics may either be simulated using traditional compartmental pharmacokinetics (with up to three compartments) or a physiologically based pharmacokinetic (PBPK) model that divides the human body into virtual organs (liver, kidneys, adipose tissue, etc.) in order to simulate realistic blood flow and distribution into tissues. In addition, depending on the specific API, metabolism via enzymes, uptake or secretion via transporters or chemical degradation can be represented in the model.

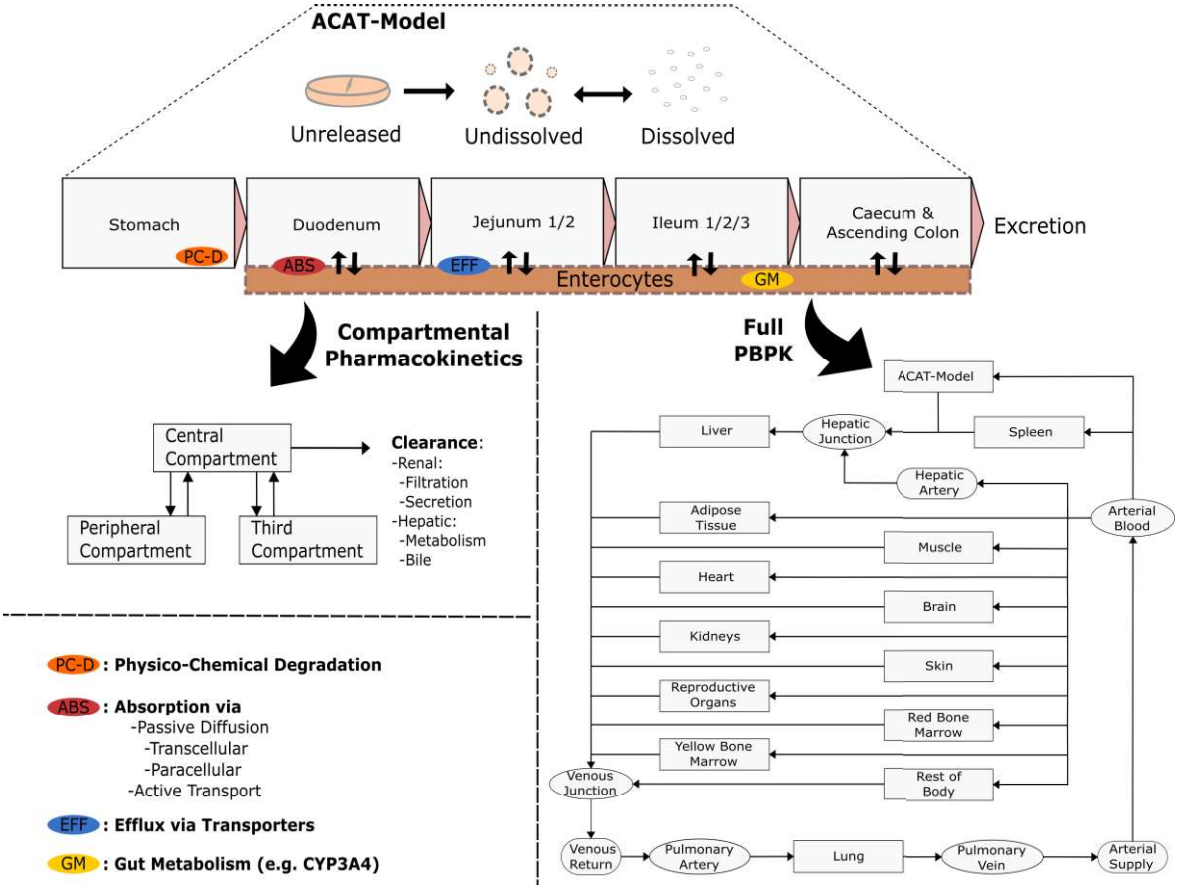

***FIGURE 6:** Schematic setup of the GastroPlus® model for simulation of drug release, gastro-intestinal transit and absorption and post-absorptive pharmacokinetics.*

The potential applications of such modelling software are numerous and range from extrapolating the *in vivo* bioavailability from *in vitro* or pre-clinical data during drug development[167], through studying potential drug-drug-interactions[168], to mechanistic pharmacokinetic investigations[169] or the conduct of virtual bioequivalence trials[170].

Despite the great potential of PBBM and PBPK models, they are still rarely used in the interaction between the PE and regulatory authorities during the drug approval process for aspects other than drug-drug interactions and pharmacogenetics[171], possibly due to a lack of standardization in model setup and validation. Simulation results are thus in most cases regarded solely as supplementary data by the regulatory authorities, to support the data sets required in the dossier for drug approval, for example in aspects regarding quality specifications for the dosage form[172] or the decision of bioequivalence to a comparator product.

Enabling the interpretation of the *in vivo* relevance of results obtained from *in vitro* tests such as the compendial quality control tests presented in the next section, modelling and simulation approaches could become indispensable tools for evaluating a drug product's performance.

2.4. Regulatory Aspects of *in vitro* Drug Release

2.4.1. Dissolution and Disintegration Testing of IR SODF in Quality Control

The European Pharmacopoeia[173] (Ph. Eur.) requires IR SODF to be tested regarding the time until complete disintegration or dissolution is achieved in addition to the uniformity of their content (or their mass variation). The compendial tests take into account some physiological aspects in a simplified manner, but do not take into account the physiology of the GIT in its entirety. The main purpose of these tests is to detect batch-to-batch differences and to assess the general suitability of the dosage form to release its content over the course of several minutes to ensure reproducible pharmacokinetics and the desired therapeutic effect after administration.

Disintegration of SODFs is assessed according to *Ph. Eur. Chapter 2.9.1 - Disintegration of tablets and capsules*[110]. A total of six dosage form units are tested, placing each unit in a cylindrical tube (with a sieve bottom) located in a basket-rack assembly (FIG. 7). A plastic disc is added to each tube to prevent dislocation of the dosage form unit during the experiment. The assembly is then submerged in water (hydrochloric acid or SGF are alternatives for coated tablets and capsules) with a temperature of 37 ± 2°C and reciprocated vertically with 29 - 32 dips per minute. After 15 min (uncoated tablets) or 30 min (coated tablets, capsules), the tubes are visually inspected. The dosage forms pass the test when all dosage forms have completely disintegrated within the specified time period. While the test is simple to perform, its physiological relevance has been questioned, as pressure profiles recorded with a SmartPill® device revealed that the compendial test does not adequately reflect pressure spikes occurring during late phases of gastric emptying[174].

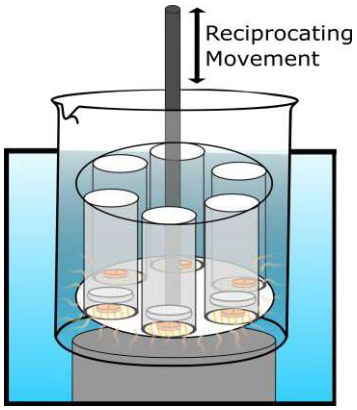

FIGURE 7: *Schematic operation of a compendial disintegration tester.*

Dissolution testing for IR dosage forms according to *Ph. Eur. Chapter 2.9.3 – Dissolution test for solid dosage forms*[126] is usually conducted in the USP apparatus I or II. However, USP apparatus III (reciprocating cylinder) or IV (flow-through cell) may also be used, especially when changing the dissolution medium during the test is desired. For the USP apparatus I and

II, media volumes of 500 – 1000 mL are recommended, with the aim of assessing dissolution under *sink conditions*, so that the concentration of dissolved API does not influence the release rate of undissolved API. The dissolution testers are usually operated at 50 – 100 RPM and a temperature of 37 ± 0.5°C. Dissolution media recommended in the Ph. Eur. exhibit a pH in the range of 1 - 8 and can be classified as *Level 0 or 1* media in the classification system of Markopoulos and co-workers[136]. The sampling time points, dissolution media composition, in-process changes in media, exact rotational speed and volume all have to be specified, taking into account the individual physicochemical properties of the dosage form in order to establish test conditions that can reliably detect significant batch-to-batch variability. The compendial release test is passed at the first test level when each of six individually tested dosage forms released 80% or more of the label API content over the specified time interval (usually less than 45 min).

In addition to fulfilling these compendial quality aspects, the therapeutic efficacy and safety of the drug product has to be shown in clinical trials for regulatory approval, which can, in the case of generic drug products, also be demonstrated by means of pharmacokinetic bio-equivalence or, for certain BCS class I and III drugs, solely based on *in vitro* investigations via the BCS-based biowaiver procedure.

The various approval procedures for generic drug products are presented in the following section.

2.4.2. Regulatory Approval of Generic Drug Products in Europe

2.4.2.1. Approval Types in the European Union

Approval procedures applicable to generic drug products are the centralized procedure (CP), decentralized procedure (DCP), mutual recognition procedure (MRP), and national authorisation in a single country[175]. When generic approval via CP is sought, the application is evaluated by the EMA Committee for Medicinal Products for Human Use (CHMP). In the case of a positive opinion and subsequent authorization by the European Commission, market authorization is granted to the medicinal drug product for all EU member states (as well as Norway, Iceland and Liechtenstein). When approval for multiple, but not all member states of the EU is sought, the DCP or the MRP is applied, depending on the market authorization status of the drug product. If market authorization has already been granted to the drug product in an EU member state, the MRP is applied. The country where the drug product is already authorized is designated as Reference Member State (RMS) and is commissioned to create an assessment report based on the dossier submitted by the pharmaceutical company seeking approval. The decision for approval is then made by the regulatory authorities of the other, Concerned Member States (CMS) based on the assessment of the RMS. When market

authorization for the drug product has not been granted in an EU member state yet, the DCP is applied. In that case, one of the member states is assigned as RMS and the others as CMS.

The frequency of use for the various aforementioned drug approval types is exemplified in TBL. 2 on the basis of drug approvals from 2013 – 2018 in the EU[176,177] and in Germany[178]. The vast majority (> 70%) of drug approvals in Germany originated from DCP for known substances, of which 74.1% of applications in the EU concerned generic drug products.

TABLE 2: *Frequency of use for various drug approval types from 2013-2018 in the EU and in Germany.*

	2013	2014	2015	2016	2017	2018	Sum	Rel. Share
Centralized Procedure (CP) – Positive Opinions								
Overall	81	82	93	81	92	84	**513**	**100%**
Generics*	16	5	25	22	21	9	**98**	**19.1%**
Decentralized Procedure (DCP) – Positive Opinions								
Overall	1052	797	1129	1133	1205	1023	**6339**	**100%**
Generics*	797	570	852	836	893	749	**4697**	**74.1%**
Mutual Recognition Procedure (MRP) – Positive Opinions								
Overall	207	249	217	249	310	291	**1523**	**100%**
Generics*	136	117	133	153	193	174	**906**	**59.5%**
All non-national Procedures (CP,DCP,MRP)								
Overall	1340	1128	1439	1463	1607	1398	**8375**	**100%**
Generics*	949	692	1010	1011	1107	932	**5701**	**68.1%**
*Drug Approvals in Germany***								
Overall	1770	1182	1364	1468	1463	1285	**8532**	**100%**
CP (All)	81	82	93	81	92	84	**513**	**6.0%**
New Substance (Non-CP)	21	21	97	63	1	4	**207**	**2.4%**
MRP (Known Substance)	119	53	84	93	83	62	**494**	**5.8%**
DCP (Known Substance)	1271	859	941	989	1079	949	**6088**	**71.4%**
National (Known Substance)	278	167	149	242	208	186	**1230**	**14.4%**

*Informed Consent, Well Established Use, Biosimilars and Hybrid Applications not included
**Re-/Parallel-Importation, Homoeopathy/Anthroposophy, and Phytopharmaceuticals not included
The mismatch between DCP approval numbers in Germany and positive opinions observed for some years (e.g. 2013 & 2014) is explained by the time period between the date of publication of an assessment report and the date of effective regulatory authorization.

The approval of generic drug products can therefore be regarded as a major contributor to regulatory burden. The standard procedure requires time-consuming and cost-intensive pharmacokinetic studies in humans, as therapeutic equivalence and safety relative to the respective innovator drug product needs to be assessed.

2.4.2.2. Bioequivalence Trials

Comparative pharmacokinetic studies in humans are the standard procedure recommended by regulatory authorities for demonstration of bioequivalence between drug products[3,179]. The study protocol usually consists of a randomised, 2-sequence, 2-period, crossover trial with a washout phase in-between periods. The number of healthy adult subjects enrolled in the study is calculated based on the expected intra-subject variability to ensure a sufficiently powered

study (β = 0.2). In order to reduce the intra-subject variability, the drug products (test and reference) are preferably administered in the fasted state. However, administration in the fed state may be necessary for drugs that are labeled to be administered with a meal. For the assessment of bioequivalence, the following pharmacokinetic outcome parameters are compared between the test and reference formulations: the highest observed blood plasma concentration of the drug (C_{max}), the area under the concentration-time curve (AUC) and, if of therapeutic relevance, the time period (t_{max}) from drug administration until observation of C_{max}. Geometric means of the intra-subject ratios between test and reference formulations for C_{max} and AUC as well as their respective 90% confidence intervals (CI) are calculated and must reside completely within the range of 0.8 – 1.25 in order for the formulations to be deemed bioequivalent. An example is illustrated in FIG. 8.

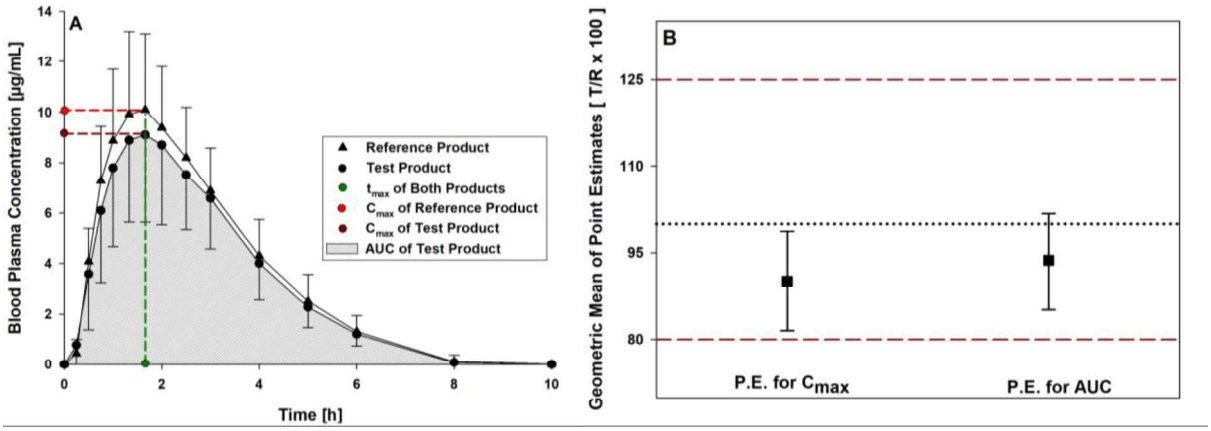

FIGURE 8: (A) *Comparison of two plasma concentration profiles illustrating C_{max}, AUC, t_{max}.* ***(B)*** *Point estimates and 90% confidence intervals for C_{max} and AUC. As both confidence intervals reside within the limits of 80.00-125.00% with no difference in t_{max}, the test product can be deemed bioequivalent to the reference product.*

2.4.2.3. BCS-based Biowaiver

Introduced in 2000 on the basis of the BCS, the BCS-based biowaiver allows for the exemption of demonstrating bioequivalence via *in vivo* bioavailability studies in favour of demonstrating similarity of dosage form performance *in vitro* for IR SODF containing BCS class I or III APIs. The approach was first implemented by the FDA[78] and was later adopted by other regulatory authorities such as the EMA[75] in 2002 or international organizations such as the WHO[180] in 2006. In the first FDA and EMA guidance documents, the BCS-based biowaiver was restricted to BCS class I compounds, as their absorption is neither limited by solubility nor permeability, and the dosage form is assumed to have no influence on oral bioavailability when dissolution is faster than gastric emptying. In principle, this is an extension of the waiver of *in vivo* bioequivalence studies for oral solutions, where bioequivalence is regarded as self-evident when two drug products contain an API in identical concentration and no excipient effects on

gastrointestinal transit, API stability and intestinal absorption are expected. Thus, when two IR SODF with BCS class I APIs dissolve completely prior to gastric emptying, their biopharmaceutical behaviour is expected to be similar to an administered solution, likewise implying self-evident bioequivalence. Further contributing to this thought process, the WHO progressively considered BCS class III and (for a brief time period) even weakly acidic BCS class II compounds eligible for a BCS-based biowaiver[180]. Their inclusion can also be explained analogous to the self-evident bioequivalence of oral solutions: for BCS class III compounds, oral bioavailability is limited due to poor permeability or saturation of active transport mechanisms. When dissolution in the stomach is very rapid, and no excipients effects altering absorption are expected, an influence of the dosage form on oral bioavailability may be ruled out. Regarding BCS class II weak acids, most exhibit poor solubility in the gastric environment, while solubility in the physiological intestinal environment is often high and not a factor limiting oral bioavailability. Therefore, although being classified as BCS II compounds based on physicochemical properties, they can be expected to behave like BCS class I compounds *in vivo*. While the inclusion of BCS class III compounds found scientific consensus[35–37] and regulatory acceptance in the revised EMA[3] and FDA[4] guidance documents, weakly acidic BCS II compounds were eventually excluded from the procedure, even in the WHO Guidance[5] that had formerly included them. Concerns were raised as to whether the dissolution setup and specifications are able to reliably discern *in vivo* bioequivalent from BCS class II drug products that are not bioequivalent, as case examples were reported in which *in vivo* bioinequivalent drug products showed similarity in the *in vitro* release test (e.g. ibuprofen[27]). To date, the potential inclusion of BCS class II compounds is still controversially discussed in the pharmaceutical literature[29–31,33,181–183].

The experimental setup for comparative release testing in the context of the BCS-based biowaiver is in large part based on compendial QC dissolution tests for SODFs. However, the details of the experimental procedure are more precisely defined, as explicit recommendations for the composition of dissolution media, fluid volumes, and rotational speeds are given.

Experimental considerations and specifications for a waiver of bioequivalence for BCS class I drugs were already proposed in the framework of the BCS. It was stated that the dosage forms have to be either very rapidly dissolving (≥ 85% release within 15 minutes, VRD) or rapidly dissolving (≥ 85% release within 30 minutes, RD) throughout the physiological pH range of 1 - 8. When both drug products are rapidly dissolving, similarity of their dissolution profiles has to be further demonstrated applying the ***f_2-Test*** described by Moore and Flanner[184] (EQ. 5):

$$f_2 = 50 \cdot Log\left\{\left[1 + \frac{1}{n_D}\sum_{t=1}^{n_D}(R_t - T_t)^2\right]^{-0.5} \cdot 100\right\} \qquad \text{(EQ. 5)}$$

Where f_2 is the dimensionless similarity factor (ranging from 0 to 100, where 100 represents congruence of both dissolution profiles), n_D is the total number of dissolution time point comparisons and R_t and T_t are the amounts released at time t for the reference and test formulation, respectively. Two dissolution profiles can be regarded similar when f_2 is ≥ 50, indicating an average absolute difference ≤ 10% among the compared dissolution time points. An example is illustrated in FIG. 9, where one drug product (Test Product A) can be deemed similar to the reference drug product (f_2 = 65.0), while the other drug product fails to comply with the BCS-based biowaiver criteria for the f_2-Test (f_2 = 41.2).

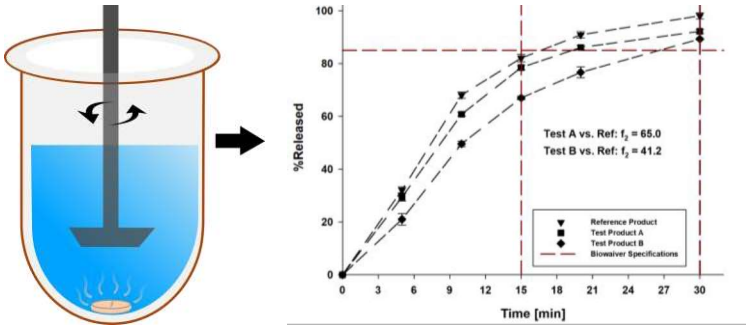

FIGURE 9: *Assessment of in vitro similarity of release profiles applying BCS-based biowaiver criteria for three rapidly dissolving drug products containing the same BCS class I API.*

For BCS class III drugs, only the VRD criterion is used in order to emphasize the need for complete dissolution before gastric emptying for permeability-limited drugs[6].

In addition to the formal prerequisites regarding the dosage form and API (same dosage form, molar dose strength, route of application and, in case of different salt forms, similar solubility), the requirements set in comparative *in vitro* release testing and data supporting a clear classification as BCS I or BCS III, an evaluation of the included excipients as well as a thorough risk-benefit analysis is expected. For BCS class I drugs, the use of well-established excipients in usual quantities is required, while for BCS class III drugs, the same excipients in similar quantity (in a defined variation range) have to be used in both drug products to be compared. Critical excipients, such as sweeteners (e.g. mannitol, sorbitol) or surfactants (e.g. polysorbates), must be assessed regarding their influence on the absorption process and be identical in quality and quantity for both drug products[6].

In a risk-benefit analysis, potential risks associated with an inappropriate biowaiver decision (i.e. drug products are deemed similar *in vitro*, but are in fact not bioequivalent *in vivo*) are to be discussed. The analysis should take into account particularities of the drug's oral absorption (e.g. absorption windows, saturable active transport and excipient effects), and the impact of sub-/supra-bioavailability on the drug's efficacy and toxicity[5].

3. Aims of the Thesis

Since its introduction two decades ago, the procedure of the BCS-based biowaiver has been continuously re-evaluated and modified among the various authorities, and is currently in the process of international harmonization, with a revised ICH guidance coming into effect in July 2020. Previous additions to the procedure were met with both, support and criticism in the recent scientific literature. Further reflecting the current ambiguity regarding theoretical and practical considerations, the procedure has been applied with varying frequency and success in generic drug approvals.

Thus, in order to comprehensively assess and evaluate current developments and potential refinements to the procedure, four key aspects are addressed in this thesis, aiming to

1) Review the current state of the regulatory framework and the utilization frequency of the BCS-based biowaiver in generic drug approval:

   The frequency of use of the BCS-based biowaiver in regulatory drug approval over the last years is summarized in order to assess the relevance of the procedure and its potential to lower regulatory burden on generic manufacturers. Recent regulatory and scientific developments regarding the procedure are discussed on the basis of the current, harmonized ICH M9 guidance and potential hindrances to successful application in the regulatory approval of generic drugs are highlighted.

2) Assess the eligibility of APIs for the BCS-based biowaiver procedure, focusing on drugs listed on the current WHO EML:

   Solubility and permeability data available in the pharmaceutical literature are reviewed with the purpose of establishing a reliable BCS-classification for essential APIs and thus create an overview of the number of essential APIs eligible for the BCS-based biowaiver. In cases where inconclusive solubility data is observed, new experimental data are generated. Further, with the aim of establishing a publicly available database in the form of biowaiver monographs, a risk-benefit analysis recommended by the WHO to be performed in the course of the BCS-based biowaiver procedure is presented and discussed on the basis of folic acid as a case example.

3) Investigate the experimental applicability of the BCS-based biowaiver to commercially available, generic drug products containing essential APIs:

   The percentage of generic drug products containing essential APIs eligible for the BCS-based biowaiver procedure that fail to meet the required dissolution specifications is investigated by a retrospective assessment of dissolution tests performed at the Goethe University, Frankfurt. To further explore key hindrances preventing the successful *in vitro* comparison of generic drug products and the range of quality differences to be expected among generic drug products, the biopharmaceutical properties of commercial tablet formulations containing the essential APIs amoxicillin and doxycycline are characterized in *in vitro* experiments and compared on a national and international level. Potential modifications to the experimental procedure of the BCS-based biowaiver, such as the utilization of Peak Vessels™ and biorelevant media, are also investigated and discussed.

4) Examine the suitability of current BCS-based biowaiver dissolution specifications to reliably assess the biopharmaceutical impact of differences in drug quality *in vivo*:

   Results from the *in vitro* experiments are parameterized for subsequent use as input parameters in the modelling software GastroPlus®. Physiologically based biopharmaceutical models are established for oral administration of amoxicillin and doxycycline and used to compare the various generic drug products in virtual bioequivalence trials. Differences observed in the *in vitro* experiments are assessed and evaluated regarding their potential influence on *in vivo* pharmacokinetics. In combination with theoretical case scenarios, the suitability of current dissolution specifications as well as the possibility of establishing customized, API-specific dissolution specifications is evaluated.

4. Key Results and Discussion

4.1. Regulatory Application and International Harmonization of Biowaivers

4.1.1. Utilization of the BCS-Based Biowaiver in Generic Drug Approvals

Although the possibility of a generic drug approval via BCS-based biowaiver was already established in 2000 in the US and in 2002 in the European Union (EU), no significant increase in drug approvals utilizing the procedure occurred until after 2008 in the US, and, following the revision of the EMA guidance document, after 2010 in the EU (FIG. 10, modified and updated from PUBL. 3)[185–187]. With the inclusion of BCS class III drugs in the revised FDA guidance in 2017, and the harmonized ICH M9 guidance coming into effect in July 2020, a further increase in approval numbers via BCS-based biowaiver is to be expected in the future.

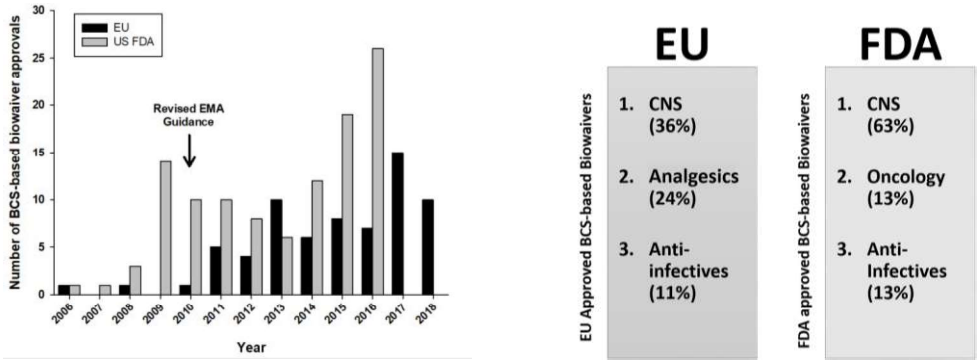

FIGURE 10: *Generic drug approvals via BCS-based biowaiver in the EU and the US (**left hand figure**) and top 3 therapeutic classes of the APIs (**right hand figures**) [modified reprint from PUBL. 3 with permission from Elsevier].*

While the average number of annual drug approvals via BCS-based biowaivers has increased over the last decade, the procedure was applied in only 6.1% of the centralized generic approvals and accounted for just 1% of all non-national generic approvals in the EU (TBL. 3).

TABLE 3: *Generic drug approvals via BCS-based biowaiver in the EU and their relative proportion in comparison to all centralized and decentralized generic approvals[185,186,177].*

	2013	2014	2015	2016	2017	2018	Sum	Rel. Share	Compared to
BCS-Based Biowaivers – Positive Opinions / (Rejected)									
CP	2	0	1	0	3 (1)	0	**6 (1)**	**6.1%**	**CP Generics**
DCP/MRP	8	6	7	7	12	10	**50**	**0.9%**	**DCP/MRP Generics**
Total	10	6	8	7	15 (1)	10	**56 (1)**	**1.0%**	**All Generics**
Potential of BCS-biowaivers exploited in CP									
Potential Candidates*	7	1	6	5	8	3	**30**	**30.6%**	**CP Generics**
BCS-Biowaivers	2	0	1	0	3	0	**6**	**20.0%**	**Biowaiver Candidates**

*Highly soluble API in SODF administered without food for systemic therapeutic effect

The lower relative share of BCS-based biowaivers in DCP (0.9%) compared to CP (6.1%) may be explained by the fact that in a DCP, each RMS and the individual CMS may have a divergent opinion regarding the overall suitability of a BCS-based biowaiver or certain aspects thereof (e.g. the therapeutic index of an API) and could thus reject the approval.

The overall low relative share in both the DCP/MRP and the CP raise the question as to whether the full potential of the BCS-based biowaiver has been exploited. In order to investigate this, all 98 APIs for which generic approval via CP was sought were evaluated regarding their eligibility for a BCS-based biowaiver based on their respective public assessment reports (PARs). Thirty of the 98 APIs were identified as highly soluble compounds formulated in IR SODF designed for a systemic therapeutic effect and administered without food. Therefore, in theory, the BCS-based biowaiver would have been applicable in 30.6% of all CP generic drug approvals, but was only applied for in 7 cases (TBL. 3).

The potential reasons for the low frequency of use are manifold and can originate from the applicant, the drug product's properties or the regulatory authority:

- **Applicant**:
    - Limited experience in the application of the BCS-based biowaiver
    - Fear of a BCS-based biowaiver being rejected by the regulatory authority (especially for BCS III compounds) which would result in delayed market access
    - Need to apply for approval in non-EU countries where a BCS-based biowaiver may not be acceptable (e.g. Japan), as approval in those countries would anyway necessitate a pharmacokinetic study in humans
- **Drug products** (generic or comparator product):
    - Inability to meet the requirements set out in *in vitro* dissolution comparison
- **Regulatory authority**:
    - Vague definition of certain criteria in the EMA guidance document (e.g. regarding allowed variations in excipients)
    - Overly strict enforcement of specifications set out in the BCS-based biowaiver guidance document

With the harmonized ICH M9 BCS-based biowaiver guidance coming into effect at the end of July 2020, it can be hoped for a positive impact on application frequency of the BCS-based biowaiver, although certain aspects that were introduced during the harmonization process may also impede the widespread use of the procedure, as discussed in the following section.

4.1.2. Harmonization of Guidance Documents

Before the international harmonization of the BCS-based biowaiver guidance documents was first pursued in July 2018[188], discrepancies among the individual guidance documents of the WHO, FDA and EMA regarding the requirements for a BCS-based biowaiver impeded its widespread use, especially when approval for a specific generic was sought after in different jurisdictions. The differences among regulatory guidance documents were evaluated in 2013[189], and, at that time, major differences identified among the guidances included the BCS classes eligible for the procedure, the approaches for solubility and permeability classification, deviating definitions for the dose to be used for BCS classification, and subtle discrepancies in the recommendations for dissolution testing. Since then, the WHO and the FDA guidance were revised and the ICH drafted a harmonized guidance document, aiming to abolish ambiguities and to facilitate generic drug approval across jurisdictions. In PUBL. 3, the current state of the BCS-based biowaiver was reviewed in order to re-evaluate remaining differences among the revised guidance documents and to critically assess decisions reflected in the harmonized ICH M9 draft guidance[188]. As the harmonized guidance was still in a draft state at the time PUBL. 3 was issued, adjustments made in the finalized guidance document are also discussed here.

In TBL. 4, the requirements for a BCS-based biowaiver are compared as laid out in the current guidance documents of the WHO, FDA, EMA as well as in the finalized ICH M9 guidance coming into effect in July 2020. Several important aspects were already consistent among the individual guidances prior to the harmonization process, such as the eligible BCS classes (I and III), solubility and permeability criteria for BCS I classification (D/S ≤ 250 mL, f_a ≥ 85%), the specifications for evaluating dissolution similarity (VRD for BCS I/III APIs or RD combined with f_2-testing for BCS I APIs) and the exclusion of drugs with a narrow therapeutic index (NTI), and were thus identically implemented in the new ICH M9 guidance. Other aspects adopted were largely based on the revised FDA guidance, as it is the most recent and detailed guidance document: sections regarding the allowed qualitative and quantitative excipient changes for BCS class III compounds in both the FDA and the ICH guidance are based on the FDA SUPAC guideline[190], and the acceptable approaches for determining an API's permeability classification have been expanded to further include permeability assays using Caco-2 cells, an approach not taken into consideration in the current EMA guidance, but well established in the FDA's guidance.

***TABLE 4:** Comparison of BCS-based biowaiver guidances of the WHO, EMA, FDA and ICH [modified reprint from PUBL. 3 with permission from Elsevier].*

Aspects	WHO (2015)[5]	FDA (2017)[4]	EMA (2010)[3]	ICH M9 Final Guidance (2019)[6]	ICH M9 Draft[188]
Solubility					
Method	Not explicitly stated	Shake-flask or other method using USP buffer solutions; n≥3 with pH verification; validated stability-indicating assay; degradation needs to be reported	Replicate determinations at each pH condition (shake-flask or other justified method); solution pH should be verified	Shake flask method or other (e.g. small-scale), n≥3, use of compendial media and validated assay, pH verification and adjustment, suitable timeframe; if degradation >10%: no classification possible; literature data can be supportive	
Temperature	37±1°C				
pH	1.2 – 6.8	1.0 – 6.8, sufficient number of pH conditions: 1 / 6.8 / pKa / pKa±1	at least 3 buffers (1.2, 4.5, 6.8 and pKa if within range)	pH 1.2 – 6.8 (1.2 / 4.5 / 6.8) + pH with lowest solubility (within range)	
Dose strength to be used	Highest single therapeutic dose	Highest dosage strength (when single dose is higher: additional information necessary)	Highest single dose	Highest single therapeutic dose (when only highest dosage strength is highly soluble: additional information necessary)	
Specification	D:S ≤ 250 mL over pH range				
Permeability					
Specification for high permeability	Extent of absorption ≥85% in mass balance or BA studies	Extent of absorption ≥85% in mass balance, BA or human intestinal perfusion studies; single method sufficient when: BA or urinary recovery ≥85%	Extent of absorption ≥85% in mass balance or BA studies, urinary and faecal recovery (including metabolites formed after absorption)	Extent of absorption ≥85% in mass balance or BA studies, urinary and faecal recovery (including metabolites formed after absorption)	
Other Acceptable Data	*In vivo* intestinal perfusion in humans	*In vivo* or *in situ* animal intestinal perfusion, *in vitro* methods using epithelial cell culture or excised intestinal tissues	None	Caco-2 assay when absorption is controlled by passive diffusion, human *in vivo* data from published literature	
Supportive Data	*In vivo* or *in situ* intestinal perfusion in animal models; epithelial cell culture assay	Demonstration of stability in GIT to support mass-balance study using compendial or simulated gastro-intestinal fluids (37°C, 1h in gastric, 3h in intestinal media), degradation >5% suggests instability	Reported BE between aqueous and solid formulations, *in vitro* permeability assays	Demonstration of stability in GIT (for mass-balance and Caco-2) with compendial or simulated gastrointestinal fluids (37°C, 1h in gastric, 3h in intestinal media), >10% degradation prevents highly permeable classification	Preclusion of permeability classification was not stated
BCS Classification					
Eligible Classes	BCS I and BCS III				
Dissolution					
Apparatus	Paddle (USP II) / basket (USP I)	Paddle (for tablets) / basket (for capsules / floating products)	Paddle / basket	Compendial apparatus: paddle / basket; use of sinkers or other approaches for coning issues	Alternative approaches to reduce coning were not specified Purified water was considered as dissolution medium at the request of the Japanese authorities
Agitation speed	75 RPM (USP II) / 100 RPM (USP I)	50 RPM, 75 RPM for coning (USP II) / 100 RPM (USP I)	50 RPM (USP II) / 100 RPM (USP I)	50 rpm (USP II) / 100 RPM (USP I, especially with observed coning)	
Dissolution media	Pharmacopoeial media: HCl solution, acetate and phosphate buffers; No surfactants, enzymes may be used if gelatine is involved	0.1 N HCl / SGF without enzymes; pH 4.5 buffer; pH 6.8 buffer / SIF without enzymes	pH 1-6.8 (at least 1.2 / 4.5 / 6.8 + observed lowest solubility); Ph. Eur. buffers recommended; no sur-factants; use of enzymes acceptable in case of gelatine in formulation; pH verification recommended	Pharmacopoeial buffers at pH 1.2 / 4.5 / 6.8. Additional investigation may be required at pH of minimum solubility, no organic solvents or surfactants; enzymes may be acceptable for capsules or tablets with gelatine coating	
Temperature	37±1°C	37±0.5°C	37±1°C	37±1°C	
Volume	≤900 mL	≤500 mL (900 mL when justified)	≤900 mL	≤900 mL (QC volumes recommended)	
Sample size	12 samples (for f_2 testing)	12 samples	12 samples, advisable to test more than a single batch	12 samples from a batch size of >100.000 units or 1/10 of production	
Sampling	e.g. 5, 10, 15, 20, 30, 45, 60 min	sufficient number, e.g. 5, 10, 15, 20 and 30 min	e.g. 10, 15, 20, 30 and 45 min	Samples should be filtered; no specific sampling time-points recommended	

f_2-test	Mean values, CV ≤20% until 10 min, ≤10% afterwards, ≥3 time-points in total (same for both formulations and zero excluded), max. 1 time-point with ≥85% dissolution	Mean values, CV ≤20% until 15 min, ≤10% afterwards, max. 1 time-point with ≥85% dissolution	CV ≤20% for first point and ≤10% afterwards; ≥3 time-points in total (zero excluded): before 15 min, at 15 min and at release close to 85%, same time points for both formulations, n=12, max. 1 time-point with ≥85% dissolution	mean values where CV ≤20% before 10 min and ≤10% afterwards, max. 1 time-point with ≥85% dissolution; ≥3 time-points in total (zero excluded), same time-points for both products	
Specification	very rapid (BCS **I**/**III**): ≥ 85% in 15 min; rapid (BCS **I**): ≥ 85% in 30 min, f_2 test (f_2 ≥ 50) for similarity of profiles				
Comparison	Comparison of test vs. reference product, no statement regarding multiple dosage strength is made			Comparison of <u>all</u> dosage strengths of the test product against the reference drug product strengths	
Excipients					
Specification	Well established excipients in usual amounts without PK interactions or influence on absorption; critical excipients, e.g. surfactants, mannitol, sorbitol must not differ qualitatively and must be quantitatively similar; BCS I: excipients present in the comparator product or in other products with MA recommended; BCS III: excipients must be qualitatively the same and quantitatively very similar to the comparator (in reference to '*WHO quality limits on allowable quantitative changes*'[191])	BCS I: Ingredients must not significantly affect absorption; recommended using excipients that are used in FDA-approved IR-SODF, quantity should be consistent with intended function, large amounts have to be justified; evaluation of critical excipients: surfactants (polysorbate 80) and sweeteners (mannitol, sorbitol); BCS III: qualitatively identical excipients; allowed changes: technical grade of excipient, Fillers±10%, Starch±6% Disintegrants±2%, Binders±1%, Ca/Mg-Stearate±0.5%, Talc±2%, Lubricants±2%, Coating±2%, Glidants±0.2%, Total Change±10% (references the FDA SUPAC Guidance)[190]	BCS I: excipients affecting BA must be qualitatively and quantitatively the same, preferably identical excipients in similar amounts; BCS III: excipients affecting BA must be qualitatively and quantitatively identical, other excipients must be qualitatively the same and quantitatively very similar; use of well-established excipients in usual amounts, discuss possible interactions affecting BE, solubility and permeability; must describe the excipients function and justify their amount; impact of critical excipients (e.g. sorbitol, mannitol, SLS) on motility, interactions with drug substance, transporters and permeability should be identified	Test product composition should mimic reference drug product; differences should be assessed for their potential to affect absorption: solubility, motility, transit time, intestinal permeability; critical excipients: sugar-alcohols (mannitol / sorbitol), surfactants (SLS); small amounts in coating are negligible. BCS I: low risk, focus on excipients affecting absorption: max. allowed change ±10% (cumulative); BCS III: same excipients (except colorants, flavour, preservatives without effect on BA); allowed changes: technical grade of excipient, excipients influencing absorption: ±10%, Fillers±10%, Starch±6%, Disintegrants±2%, Binders±1%, Ca/Mg-Stearate±0.5%, Lubricants±2%, Talc±2%, Glidants±0.2%, Coatings±2%, Total Change±10%	No cumulative difference specification was stated for excipients affecting absorption for BCS class I drugs
Risk-benefit analysis					
Further Consider-ations	Favourable risk-benefit analysis; BCS III: address extent, site and mechanism of absorption; more critical evaluation the lower oral BA is; evaluate risk of incorrect decision: sub- and supra-BA products	Not specifically mentioned	Risk of an inappropriate biowaiver decision should be more critically reviewed (e.g. site-specific absorption, risk for transport protein interactions, excipient compositions and therapeutic risks) for BCS III than for BCS I	Not specifically mentioned	
Restrictions					
	No NTI Drugs	No NTI drugs, no products designed to be absorbed in the oral cavity, Pro-drugs only eligible when meta-bolized post-absorption	No NTI drugs, no different API forms (except BCS I salt forms with similar properties), no products designed to be absorbed in the oral cavity; no modified release formulations	No NTI drugs, no products designed to be absorbed in the oral cavity or administered without water; dosage form must be identical for both products (e.g. no capsule vs. tablet!); Pro-drugs eligible only when metabolized post-absorption; drug substances have to be identical (in case of different salt forms: both have to be BCS class I)	Different salt forms were not considered eligible; pharma-ceutically equivalent dosage forms were allowed
Comments					
		Legal Basis: Regulations at 21 CFR 320 for BA/BE, 21 CFR 320.22 for biowaivers; FDC drug products eligible for BCS-based biowaiver: when containing BCS I APIs: BCS I criteria; when containing BCS I&III or BCS III APIs: BCS III criteria	Legal Basis: Directive 2001/83 EC FDC Biowaiver possible	Documentation: polymorphic form, enantiomeric purity, bioavailability or bioequivalence problems (literature surveys); applicable to IR SODF or suspensions designed to deliver the drug to the systemic circulation and FDC drug products (containing BCS I APIs: BCS I criteria; containing BCS I&III or BCS III APIs: BCS III criteria)	

Aspects that were consolidated and differ from the FDA guidance are the dose to be used for BCS classification (highest single therapeutic dose is to be used, but highest dosage strength can be adequate when dose linearity is demonstrated) and the media volume to be used in dissolution tests (≤ 900 mL instead of the preferred ≤ 500 mL stated in the FDA guidance).

Interestingly, in addition to resolving ambiguities and the consideration of valid alternative approaches (e.g. regarding the dose to be used for BCS classification), new aspects have been introduced in the harmonized guidance that were not taken into consideration in any of the other current guidance documents. These are:

A) Raising the limit for significant degradation to an extent of ≥ 10% (compared to ≥ 5% as stated in the FDA guidance). Significant degradation is stated to prevent solubility and permeability classification and thus denies application of the BCS-based biowaiver. In comparison, the FDA guidance simply states that significant degradation needs to be reported and discussed in a BCS-based biowaiver application.
B) When an approval for multiple dosage strengths is sought, each individual strength of the test product has to be compared to the respective comparator drug product.
C) The dosage forms that are to be compared have to be identical, meaning that tablets and capsules are not considered to be similar dosage forms in the harmonized guidance. However, tablets with a coating not intended to change the release kinetics (e.g. taste-masking coatings) may be compared to uncoated tablets.
D) Suspensions are considered eligible for a BCS-based biowaiver. However, no explicit statement is made regarding allowed qualitative and quantitative excipient variations, as the relevant paragraphs and tables exclusively cover excipients used in solid oral dosage forms.
E) For solubility classification, the pH where lowest solubility is expected needs to be included in the solubility determination (if within range). In the current EMA and FDA guidances, investigation of the solubility at pH = pK_a (in addition to $pK_a \pm 1$ in the FDA guidance) is required.
F) Explicit recommendation to use the USP I apparatus at 100 RPM in the case of coning instead of increasing the RPM to 75 in the USP II apparatus. No recommendations regarding sampling time-points are made, although the individual guidance documents state such (TBL. 4).

Specifically, the additions *A)-C)* neither facilitate the procedure nor reduce the regulatory burden, as they either add criteria for a possible exclusion of APIs *(A)* and dosage forms *(C)* or make the procedure more laborious *(B)*. This seems counter-intuitive to the purpose of the BCS-based biowaiver, especially compared to other, more progressive changes that were made, such as the novel inclusion of suspensions *(D)* or the possibility of establishing the

permeability classification solely based on *in vitro* experiments (e.g. in Caco-2 assays), which was formerly not possible in the EMA guidance.

The replacement of the requirement to determine the solubility at pH = pK_a in favour of the pH where lowest solubility is expected *(E)* is much appreciated, as according to the Henderson-Hasselbalch equation, the lowest solubility of a monoprotic acid or base is not expected at pH = pK_a. In case of multiple pK_a values, the proportion of each molecule species existing at a certain pH needs to be taken into consideration in order to calculate the pH where lowest solubility is expected, but the resulting pH being identical to one of the pK_a-values is highly unlikely. Thus, scientifically, the general requirement to include the pH where the lowest solubility is expected is preferable compared to mandatory testing at pH values around the pK_a.

The recommendation to use the USP I apparatus at 100 RPM in cases where noticeable coning is observed *(F)* is surprising, as in the FDA guidance, increasing the rotational speed to 75 RPM in the USP II apparatus is stated as an alternative and is even recommended as the standard rotational speed by the WHO. The use of other experimental setups that are specifically designed to reduce coning without the need for a drastic increase in the rotational speed (e.g. the use of Peak Vessels™) are not explicitly mentioned. However, in view of these possibilities, the respective section in the ICH M9 draft guidances was changed to allow alternative experimental approaches when scientifically justified.

Compared to the draft version, most of the changes made in the finalized ICH M9 guidance (see last column in TBL. 4) further clarify individual specifications, such as the permitted cumulative change in excipients potentially affecting absorption for BCS class I drugs, or allowance for more flexibility in the approach, as alternative experimental setups for the reduction of coning may be used and different salt forms of BCS class I drugs are considered to be eligible candidates for a BCS-based biowaiver. The aforementioned exclusion of different dosage forms for the BCS-based biowaiver, however, is incomprehensible, as the various IR SODF were explicitly considered to be identical in the M9 draft guidance and still are regarded as such in the EMA guidance. This further unnecessarily limits the applicability of the BCS-based biowaiver for highly soluble drugs.

4.2. Eligibility of Essential APIs for the BCS-Based Biowaiver

The finalized ICH guidance has harmonized the fundamental criterion for the eligibility of APIs for a BCS-based biowaiver: a classification as *highly soluble* in the framework of the BCS. To assess the proportion of potential candidates among essential medicines for the procedure, publicly accessible, reliable data to support a BCS classification, especially solubility data, were generated and summarized for several essential APIs (PUBL. 1). In addition, a thorough risk-benefit assessment for an individual API (folic acid) in the form of a biowaiver monograph (PUBL. 2) was performed with the aim of contributing to a sound scientific basis for the application of the BCS-based biowaiver.

4.2.1. BCS Classification Based on Experimental Solubility Data

Sixteen APIs were identified in PUBL. 1 that were either added to the WHO EML after the 14th version[192] (and thus after the comprehensive BCS-classification assessment performed by Lindenberg et al.[23]) or for which a reliable solubility classification over the physiologically relevant pH range at 37°C had not been established yet, as the solubility of many APIs is often reported simply as their aqueous solubility at room temperature. The aim was further to report actual values for the experimental solubility at each pH to enable recalculation of the D/S and the resulting BCS solubility classification in cases where differences in the recommended dosage form strength occurred. This was considered necessary, as actual solubility values are seldom reported along with the BCS classification of an API, preventing exact assessment of the solubility classification across different dose definitions (e.g. highest single dose vs. highest dosage form strength[193]) or changes in the clinically utilized or marketed dose range. FIG. 11 depicts the D/S of the APIs based on their highest dosage strength listed on the EML. Of the investigated APIs, nine were classified as highly soluble (FIG. 11A) and are thus eligible candidates for a BCS based biowaiver, while the other seven were deemed not highly soluble (FIG. 11B), mostly due to their lowest solubility being observed at pH values reflecting the small intestinal environment (pH 6.8).

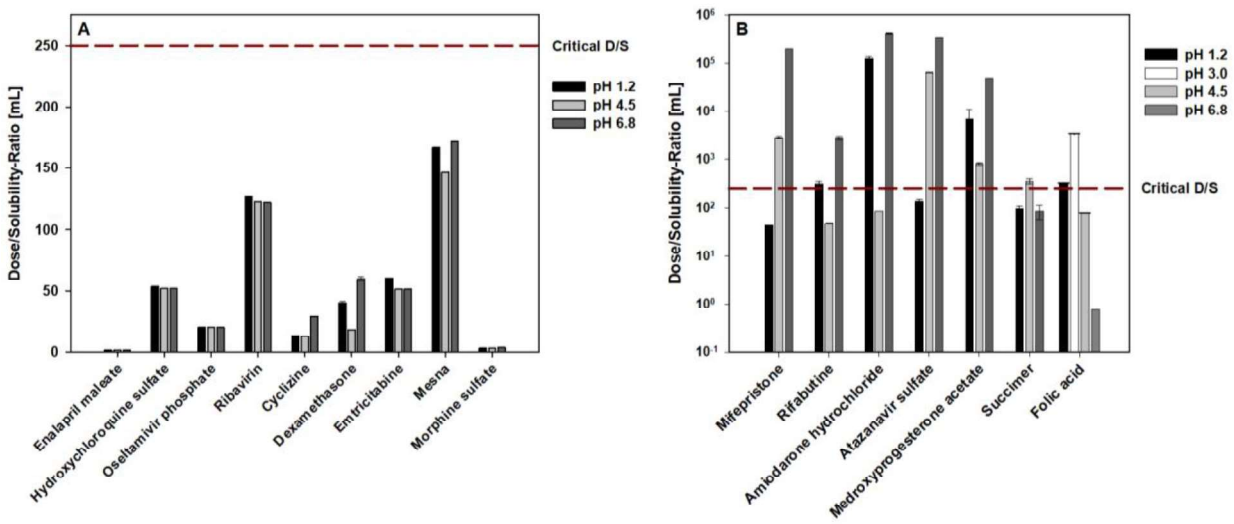

FIGURE 11: *Calculated dose/solubility-ratios for the highest dosage form strength of APIs listed on the WHO EML.* ***(A)*** *Compounds with a D/S ≤ 250 mL over the physiological pH range (1.2 - 6.8)* ***(B)*** *compounds exceeding the critical D/S at specific pH values [reprinted from PUBL. 1 with permission from Elsevier].*

However, for two APIs, poor solubility was observed at pH 4.5 (succimer) or pH 3.0 (folic acid), respectively. Regarding succimer, significant degradation was observed in addition to its borderline poor solubility, which definitely excludes this compound from being eligible for a BCS based biowaiver on the basis of the finalized ICH M9 guidance.

As discussed in PUBL. 2, folic acid is an excellent case example, demonstrating the necessity for including the pH value where the lowest solubility is expected in solubility determinations. Relying solely on its water solubility or the solubility at the recommended pH values (1.2, 4.5, 6.8), folic acid was formerly reported as a BCS class I/III API[20]. While its revised solubility classification now formally prevents an approval via BCS-based biowaiver according to the current guidance criteria, other approaches for an abbreviated approval are feasible (PUBL. 2) and are briefly discussed in subchapter 4.2.2.

By combining the experimental solubility data generated in PUBL. 1 with existing solubility data in the pharmaceutical literature and assessment reports of the regulatory authorities (EMA[185], FDA[194] and WHO[20]), a comprehensive solubility classification was established for APIs in IR SODF listed on the 20th EML as part of PUBL. 3, in order to assess the potential for application of the BCS-based biowaiver in the approval of generic drug products containing these essential APIs. As the WHO issued the 21st EML in the time period between PUBL. 3 and this dissertation, the data from PUBL. 3 was updated for this dissertation to additionally account for APIs introduced on (or deleted from) the revised list.

The 21st WHO EML includes a total of 460 medicines[11]. 175 of these are administered as single API, IR SODF with systemic effect (excluding vitamins and minerals) and are thus potential candidates for a BCS-based biowaiver. An overview of their solubility classification is given in FIG. 12A (modified and updated from PUBL. 3).

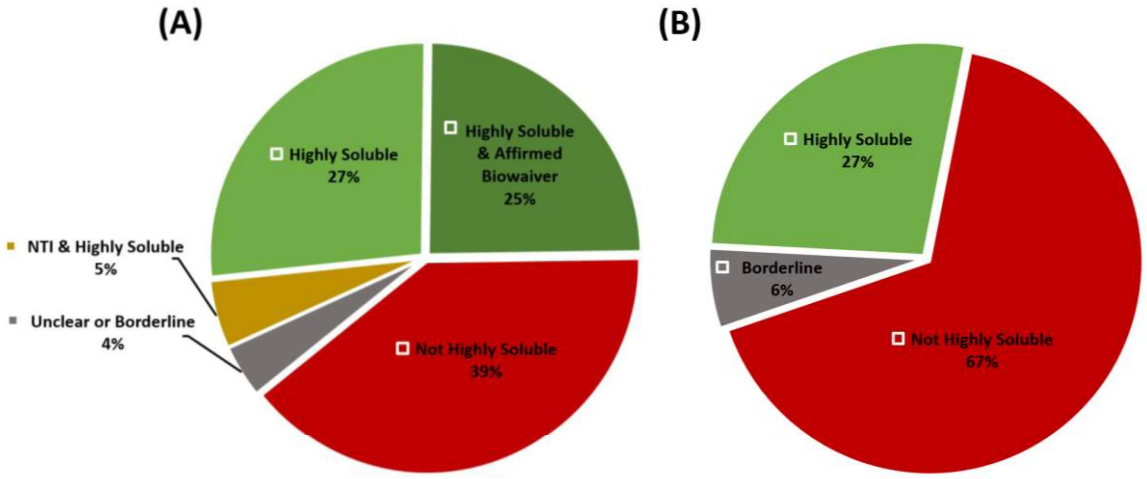

FIGURE 12: *Solubility classification of APIs listed on the 21st WHO EML and formulated in SODF designed to achieve systemic therapeutic efficacy (vitamins and mineral supplements were excluded).* ***(A)*** *Formulations containing a single API (n = 175)* ***(B)*** *Fixed-dose-combinations (n = 33) [modified reprint from PUBL. 3 with permission from Elsevier].*

Based on the highest dosage strength listed on the EML, more than half of the APIs are classified as highly soluble, even when excluding drugs with an NTI. In addition, of the 33 FDCs of essential APIs that are formulated as IR SODF, nine exclusively contain highly soluble APIs, and are thus eligible candidates for a BCS-based biowaiver (FIG. 12B).

In addition to being eligible candidates due to their solubility classification, 15 essential APIs were identified that have already been approved via BCS-based biowaivers in the EU[185,186,195] or the US[194], are recommended for the procedure by the WHO prequalification team for medicines (PQTm)[196] or may be approved based on *in vitro* experiments on the basis of FDA product guidelines[197]. A further 28 APIs received a positive opinion in the risk/benefit assessment performed in biowaiver monographs[22], so that, as a result, waiving *in vivo* bioequivalence studies in favour of an approval via BCS-based biowaiver or other *in vitro* studies is currently supported on a scientific and/or regulatory basis for 43 (~25%) of the 175 essential APIs administered as SODF (FIG. 12A).

4.2.2. Risk/Benefit-Assessment in Biowaiver Monographs

While classification as highly soluble is one formal requirement for a BCS-based biowaiver, the eligibility of the procedure is further tied to the ability of the dosage form to meet the dissolution criteria as well as a positive risk-benefit assessment. The WHO recommends taking into account the potential risks emerging from an incorrect biowaiver decision (i.e. the test product being supra- or sub-bioavailable, respectively, compared to the originator product). Further, the therapeutic index of the drug needs to be evaluated and, especially in the case of BCS class III drugs, the extent, site and mechanism of absorption is to be discussed. Addressing the aforementioned aspects, the FIP focus group for BCS and biowaiver initiated by Dr. Dirk Barends in 2004[198] has issued 48 biowaiver monographs to date (URL: https://www.fip.org/bcs-monographs). 28 of these monographs conclude a positive opinion for the application of the BCS-based biowaiver for the highly soluble, essential APIs they are concerned with. There are, however, also cases of negative opinions for highly soluble drugs evaluated in biowaiver monographs: for example, quinine and ribavirin are not recommended for approval via BCS-based biowaiver due to their narrow therapeutic index.

While the requirements for a BCS-based biowaiver put forth in the regulatory guidance documents are important to the risk assessment, the biowaiver monographs are intended to further evaluate the risk, based on biopharmaceutical and pharmacokinetic properties of APIs and their formulations, going beyond the criteria in the guidelines. Thus, the recommendations in the biowaiver monographs sometimes contradict the formal requirements: in four cases, a positive opinion was concluded despite an API classification as poorly soluble. All of these APIs are weakly acidic, anti-inflammatory drugs (diclofenac, ibuprofen, ketoprofen and piroxicam) that are highly permeable and highly soluble in the small intestine. Solely their solubility in the gastric compartment prevents their formal eligibility for the procedure. Taking into account their biopharmaceutical behaviour, it was concluded that these BCS class II drugs would demonstrate a *BCS-Class-I*-like behaviour *in vivo*, resulting in a concluded low risk for dosage form related bioavailability problems and thus a positive opinion for application of the BCS-based biowaiver.

In the biowaiver monograph for folic acid (PUBL. 2), it was similarly concluded that the overall risk of differences in the *in vivo* dissolution behaviour having an influence on the therapeutic efficacy can be considered as low, despite the drug being formally ineligible (as a BCS class II/IV compound) for a BCS-based biowaiver and the observed inability of tested dosage forms to meet the experimental dissolution requirements[199]. Toxicity is not expected to occur if the product is mildly supra-bioavailable, as no substantial side-effects were observed even when high doses (15 mg) were administered to human subjects[200], and the reported LD_{50} of 10 g/kg[201] after oral administration of folic acid in mice clearly exceeds the dose usually administered to humans (5 mg). Further, therapeutic efficacy of folic acid is tied to the metabolic capacity of the human body for conversion of folic acid to the physiologically active L-5-methyltetrahydrofolate[202]: unmetabolized folic acid appears in the systemic circulation at doses exceeding ~280 µg due to saturation of the metabolism[203,204], resulting in a large fraction of an administered dose of 5 mg folic acid being excreted unchanged[205,206]. A decrease in therapeutic efficacy is therefore not expected, even with sub-bioavailable drug products.

As even large differences in dosage form performance are unlikely to have an effect on the therapeutic efficacy, the BCS-based biowaiver approach of assessing the dissolution similarity as a surrogate for bioequivalence (and therefore also therapeutic efficacy) seems methodically unsuitable for folic acid, especially since its poor aqueous solubility at pH 3.0 would preclude drug products from meeting the dissolution requirements anyway.

In that special case, other approaches for waiving *in vivo* bioequivalence are preferable, e.g. market authorization as an “approval exempt standard formulation” (*Standardzulassung*[207]) which is possible in Germany for folic acid and other APIs (e.g. paracetamol) or medicines (e.g. medicinal tea products) which are not expected to pose a risk to public health[208]. As long as the drug product is manufactured and tested according to a corresponding official monograph, no bioequivalence testing is necessary for market authorization.

4.3. Experimental Applicability of the BCS-Based Biowaiver to Generic Drug Products

As substantiated in the first two subprojects, the BCS-based biowaiver is not exploited to its full potential in drug approvals although numerous essential and non-essential APIs are eligible candidates for a BCS-based biowaiver. To further investigate one of the postulated reasons for this, the *in vitro* performance of drug products containing various essential APIs was examined with the aim of assessing the failure rate and identifying practical obstacles preventing the successful application of the procedure (PUBL. 3 and PUBL. 6).

4.3.1. Assessment of the Failure Rate of SODF in Dissolution Comparisons

Several cases were found in the literature where drug products containing highly soluble APIs failed to comply with the BCS-based biowaiver dissolution requirements[24,28,209,210].

To substantiate these observations and to estimate the failure rate of the BCS-based biowaiver, results from dissolution experiments on drug products containing essential APIs conducted at the Goethe University, Frankfurt am Main, were summarized and evaluated retrospectively. The outcome of the assessment is depicted in FIG. 13.

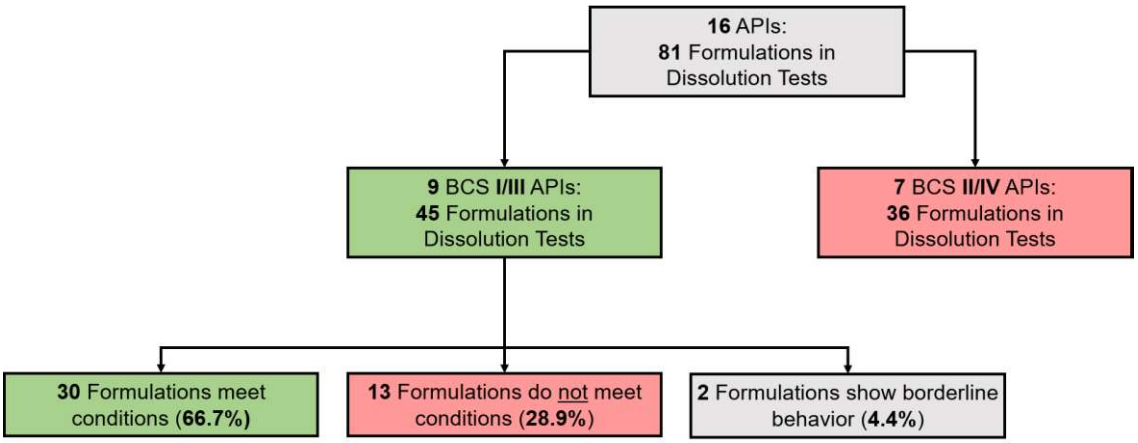

Figure 13: *Outcome of dissolution tests performed with essential medicines applying BCS-based biowaiver specifications for demonstration of similarity in vitro [modified reprint from PUBL. 3 with permission from Elsevier].*

Eighty-one formulations containing essential APIs in IR SODF were identified that had been subjected to BCS-based biowaiver conform dissolution tests. As expected, none of the 36 formulations containing BCS class II/IV APIs were able to release ≥ 85% of their content within 30 minutes under all imposed dissolution conditions (pH 1.2, 4.5, 6.8) but surprisingly, a third of the dosage forms containing highly soluble APIs were also not able to meet the specifications, with highest failure rates observed for drug products containing doxycycline (3/9), ethambutol (4/7) and chloroquine (2/3).

Compared to the observed low failure rate of BCS class I and III APIs in bioequivalence trials[211] (~13% overall, ~5.1% in sufficiently powered studies), the BCS-based biowaiver seems to be over-discriminating, which greatly reduces its utility.

While a point could be made that an overly-discriminative procedure is favourable in regard to patient safety, examples from the literature where *in vivo* non-bioequivalent drug products showed dissolution similarity *in vitro* suggested that the stricter conditions do not necessarily prevent false-positive test outcomes[25,26,212]. However, the validity of some of these *in vitro* – *in vivo* comparisons is to be questioned. Ramirez et al. compared data from regular quality control methods instead of BCS-based biowaiver conform dissolution tests[212], and in one of the case examples, Ketoprofen is assigned as BCS class I[26], whereas in other publications, it is classified as BCS class II compound[213]. It is unclear whether the reported discrepancies would still have occurred while correctly applying the BCS-based biowaiver procedure. Nonetheless, this raises the question as to whether the current regulatory requirements regarding the experimental dissolution setup and the 'one size fits all' specifications are adequate tools for the reliable assessment of the influence of differences in dosage form performance on a drug's pharmacokinetic profile.

To assess the magnitude of differences in dosage form performance among various generics on an international and national level, IR SODF of drug products with a high failure rate observed in the retrospective assessment or reported in the literature were obtained and subjected to the BCS-based biowaiver procedure.

Doxycycline and amoxicillin were chosen as model drugs for this investigation. Doxycycline generics exhibited a high failure rate in the retrospective assessment, while drug products containing amoxicillin were reported by Reddy et al.[24] and Löbenberg et al.[214] to frequently fail to comply with the BCS-based biowaiver criteria. Indeed, one case was reported where drug products manufactured and marketed by the same pharmaceutical company in different countries failed to demonstrate similarity *in vitro*[214].

Despite similar BCS classification, the biopharmaceutical and pharmacokinetic properties of both APIs show marked differences, as summarized in TABLE 5. This circumstance further contributes to the APIs being suitable candidates for a more detailed investigation of the practical applicability and the discriminatory power of the BCS-based biowaiver in regard to potential effects of differences in dosage form performance on the resulting pharmacokinetics of a drug product.

TABLE 5: *Comparison of physico-chemical, biopharmaceutical and pharmacokinetic properties of amoxicillin and doxycycline.*

Parameter	Amoxicillin	Doxycycline
Salt form	Trihydrate	Monohydrate (MH) or Hyclate (H)
Highest Dose on EML [mg][11]	500	200
BCS Class	I (≤ 750 mg)[215]	I/II (MH), I (H)[216]
pK_a	2.67 / 7.11 / 9.55[217]	3.02 / 7.97 / 9.15[218]
Log P	0.87[219]	-0.2[220]
Absorption	Active transport (via hPEPT) in proximal small intestine[221–223]	Moderate passive Diffusion (P_{app} = 17.5 x 10^{-6} cm/s)[224] with EHC[225]
T_{max} [h]	1 – 2[215]	1.5 – 3.5[220]
Elimination half-life [h]	1 – 1.5[226–228]	12 – 25[220]

4.3.2. International Level – Dissolution of South African and German Drug Products

Substantiating the findings of Reddy et al.[24] and Löbenberg et al.[214], two drug products containing amoxicillin in combination with clavulanic acid obtained from the German and South African market could not demonstrate similarity of their dissolution profiles under BCS-based biowaiver conditions (FIG. 14, mod. from PUBL. 4), although they were manufactured and distributed by the same pharmaceutical company (AUROBINDO PHARMA LTD, Hyderabad, India).

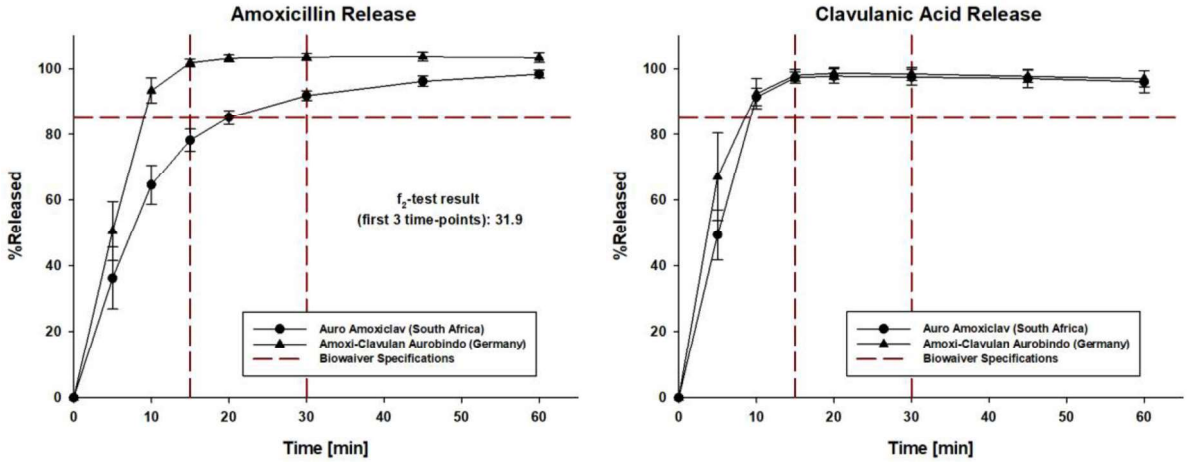

Figure 14: *Release profiles of a German (AMOXI-CLAVULAN AUROBINDO) and a South African (AURO AMOXICLAV) generic drug product containing amoxicillin and clavulanic acid in 900 mL SIF_{sp} at pH 6.8, 37°C and 75 RPM in the USP II apparatus.* ***Left hand figure****: release profiles for amoxicillin.* ***Right hand figure****: release profiles for clavulanic acid. Error bars indicate standard deviations (n=5) [modified reprint from PUBL. 4 with permission from John Wiley and Sons].*

While the release profiles for clavulanic acid are almost superimposable and reach complete dissolution within 15 minutes, dissolution of amoxicillin was very rapid (< 15 min) for the German drug product, but only rapid (< 30 min) for the South African drug product and could thus not be deemed similar applying the BCS-based biowaiver criteria. This variation in drug quality and, more specifically, dosage form performance for drug products from the same pharmaceutical company is surprising and raises the question regarding the interchangeability of international drug products. A possible explanation for the discrepancy among international drug products is the fact that generic drug products for different countries are usually tailored to local comparator products[214]. While this seems necessary from a practical point of view, it poses an obstacle for global harmonization of drug quality and thus the widespread application of the BCS-based biowaiver when market authorization for generics in different countries is sought.

Against this background, the WHO initiative for harmonization of international comparator drug products[229,230] constitutes an excellent solution for the aforementioned lack of harmonization. The WHO proposed international comparator list can ensure equal international quality standards and can facilitate both the conduction of *in vivo* bioequivalence trials as well as BCS-based biowaivers, as comparison against multiple comparator products for different countries would no longer be necessary, and generics could be tailored to specific, well-characterized comparator drug products.

4.3.3. National Level – Dissolution Performance of German Generics

To further investigate quality differences on a national level, the applicability of the BCS-based biowaiver to generic drug products containing amoxicillin or doxycycline available on the German market was evaluated in PUBL. 6. Five commercial tablet formulations of each API were obtained that are interchangeable in German public healthcare and can thus be assumed bioequivalent. The biopharmaceutical characteristics of the drug products were evaluated in compendial quality control tests, BCS-based biowaiver conform dissolution tests and biorelevant *in vitro* methods in order to investigate the range of dosage form performance differences of bioequivalent drug products, their false-negative rate in the BCS-based biowaiver as well as problems preventing the successful application of the procedure.

In compendial disintegration tests using media of pH 1.2 – 6.8, great variability was observed in the time needed for complete disintegration of the drug products (FIG. 15).

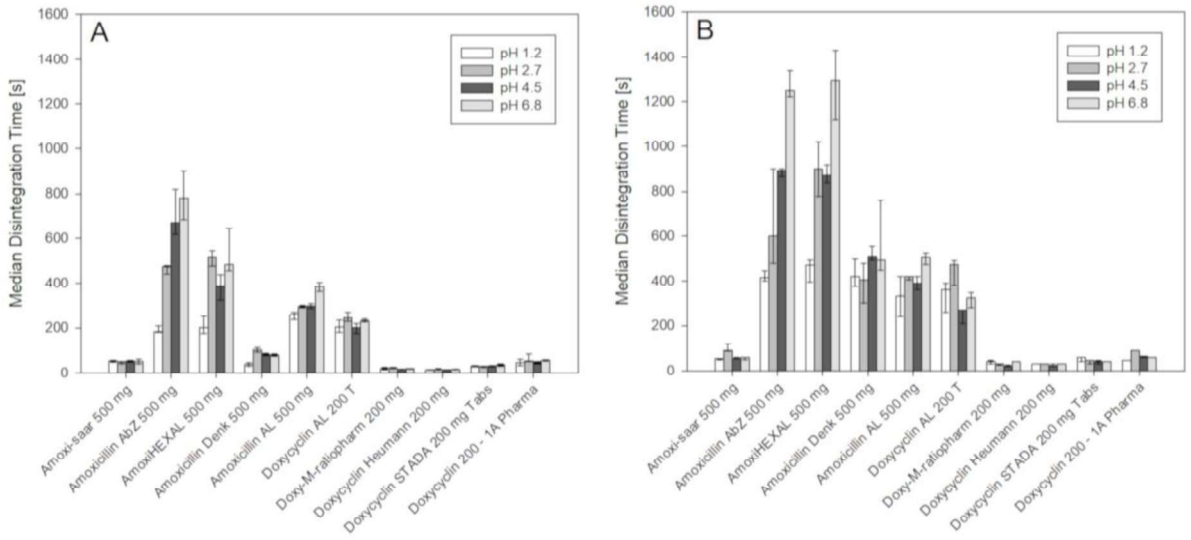

Figure 15: *Median disintegration times of dosage forms containing amoxicillin or doxycycline at various media pH values observed in* ***(A)*** *compendial disintegration testing* ***(B)*** *dissolution testing in the USP II apparatus (50 RPM). Error bars indicate the observed range (n=3) [reprinted from* PUBL. *6 with permission from Elsevier].*

For most products containing amoxicillin, disintegration was found to be dependent on media pH, with the fastest disintegration times being observed at lower pH values reflecting the gastric compartment. In contrast, the drug products containing doxycycline were found robust to changes in media pH, and demonstrated overall rapid disintegration. Further, when the disintegration time was investigated in the USP II apparatus, most amoxicillin drug products exhibited a noticeable increase in disintegration time, with the exception being AMOXI-SAAR, which proved to be very robust to changes in media pH as well as the experimental setup used, similar to the doxycycline products. This implies that for the amoxicillin drug products, disintegration is expected to be a major factor contributing to the successful application of the BCS-based biowaiver, while for the doxycycline products, the drug particle dissolution rate is expected to be crucial, as disintegration was observed to be very rapid and robust to external conditions.

Results from dissolution experiments obtained with the USP II apparatus using 500 mL of media (pH 1.2 – 6.8) at 37±0.5°C, *Peak Vessels*^{TM} and 50 RPM (or 75 RPM when biowaiver specifications could not be met) confirmed the aspects most likely hindering the successful application of the BCS-based biowaiver (FIG. 16).

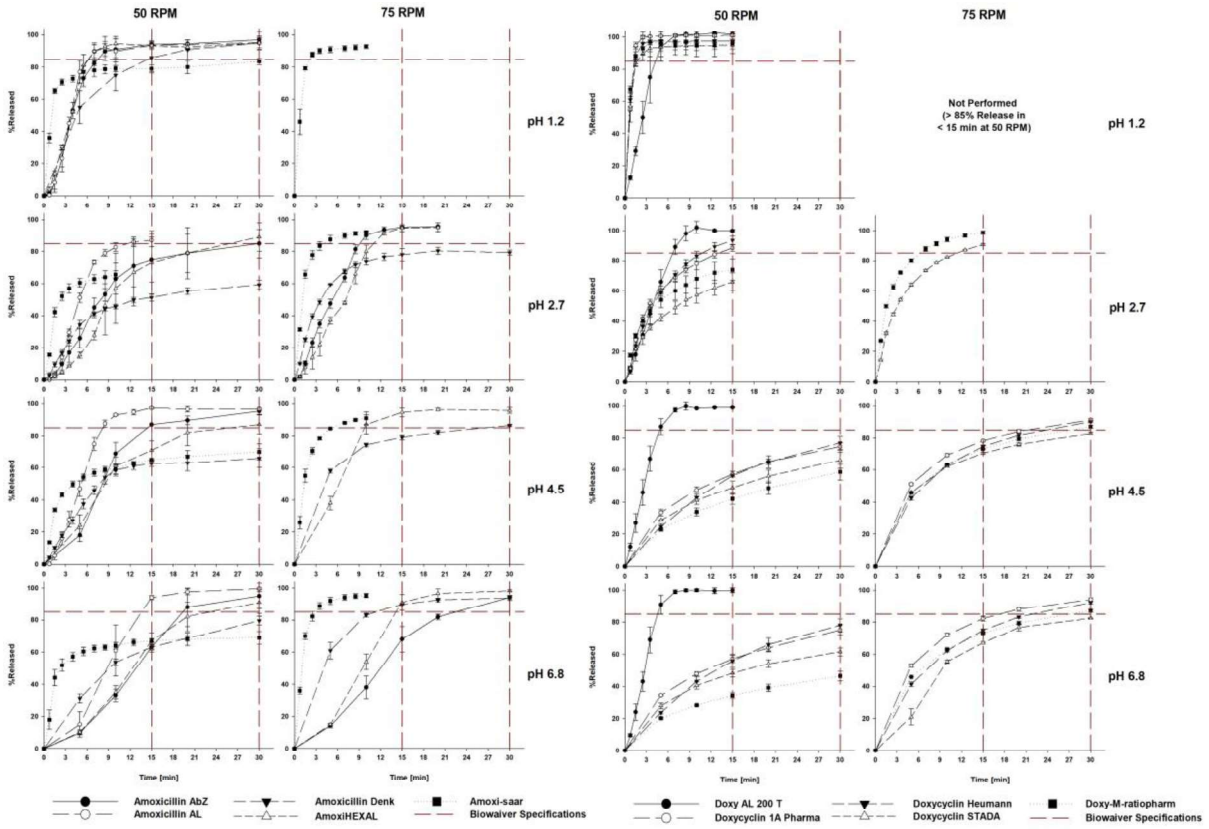

FIGURE 16: *Release profiles of SODF containing amoxicillin (**left hand figures**) or doxycycline (**right hand figures**) using Peak Vessels™ in the USP II apparatus and 500 mL of dissolution media at 37°C. Error bars depict standard deviations (n=3) [reprinted from PUBL. 6 with permission from Elsevier].*

Long disintegration times led to a large variability among the amoxicillin drug products (especially at higher pH values), while pH-dependent particle dissolution was the predominant characteristic for doxycycline monohydrate tablets. In addition, for most drug products, occurrence of coning was identified as a major confounding factor to the ability to reach ≥ 85% release, and could not be completely prevented even when using *Peak Vessels™* and 75 RPM.

The observed differences in dissolution profiles were evaluated applying the BCS-based biowaiver criteria, comparing four drug products of each API to the generic product first introduced to the German market as the comparator, since the innovator product was no longer available for either API. Results are summarized in TBL. 6.

TABLE 6: *Possibility of demonstrating in vitro similarity among generics containing amoxicillin or doxycycline when applying BCS-based biowaiver dissolution specifications [modified reprint from PUBL. 6 with permission from Elsevier].*

Comparator Product	Test Product	VRD or RD Criteria Fulfilled for Comparison?	f_2-Test Result	Demonstration of Equivalence Possible *in vitro*?
AMOXICILLIN AL 500 MG	AMOXI-SAAR 500 MG	VRD (75 RPM / pH 1.2 - 6.8)	N/A	Yes
	AMOXIHEXAL 500 MG FILMTABLETTEN	VRD (50 RPM / pH 1.2 and 75 RPM / pH 2.7 - 6.8)	N/A	Yes
	AMOXICILLIN ABZ 500MG FILMTABLETTEN	VRD (75 RPM / pH 1.2 - 4.5 and FaSSIF-V3) RD (50 RPM / pH 6.8) + f_2-Test	pH 6.8: 33.3	No (compendial media)
	AMOXICILLIN DENK 500 MG TABLETTEN	Test Product fails to meet RD criteria (75 RPM / pH 2.7)	N/A	No
DOXYCYCLIN HEUMANN 200 MG	DOXYCYCLIN 200 1A PHARMA	VRD (50 RPM / pH 1.2 - 2.7) RD (75 RPM / pH 4.5 - 6.8) + f_2-Test	pH 4.5: 64.0 pH 6.8: 52.6	Yes
	DOXY-M-RATIOPHARM 200 MG	VRD (50 RPM / pH 1.2 and 75 RPM / pH 2.7) RD (75 RPM / pH 4.5 - 6.8) + f_2-Test	pH 4.5: 59.1 pH 6.8: 71.4	Yes
	DOXYCYCLIN STADA 200 MG TABS TABLETTEN	Test Product fails to meet RD criteria (75 RPM / pH 4.5 - 6.8)	pH 4.5: 65.2 pH 6.8: 46.9 FaSSIF-V3: 52.0	No (compendial media)
	DOXYCYCLIN AL 200 T	VRD (50 RPM / pH 1.2 - 2.7) RD (75 RPM / pH 4.5 - 6.8) + f_2-Test	N/A	No

VRD: Very rapidly dissolving (≥ 85% Release in ≤ 15 minutes) RD: Rapidly dissolving (≥ 85% Release in ≤ 30 minutes) N/A: Not applicable

Substantiating the high failure rate observed in the retrospective assessment, similarity of dissolution profiles compared to the comparator product could not be demonstrated for half of the drug products, even when using favourable conditions such as 75 RPM and *Peak Vessels*^{TM}. Specifically, slow dissolution and/or disintegration at higher pH values (pH 6.8) prevented demonstration of similarity for DOXYCYCLIN STADA and AMOXICILLIN ABZ to the respective comparator drug product. In both cases, f_2-test values < 50 were obtained in compendial SIF_{sp}.

When the biorelevant medium FaSSIF-V3 was used instead of compendial SIF_{sp}, both products that formerly failed to meet the requirements in SIF_{sp} were now able to demonstrate similarity, because dissolution was now very rapid (AMOXICILLIN ABZ) or rapid with an f_2-value > 50 (DOXYCYCLIN STADA) in the biorelevant medium, as shown in FIG. 17.

While the measured thermodynamic solubility was similar in both media, the lower surface tension in FaSSIF-V3 likely facilitated wetting of the tablets and subsequently promoted faster disintegration and dissolution. In addition, the lower concentration of buffer salts may also have had an influence on the pH in the hydrodynamic layer around drug particles, possibly resulting in an increased dissolution rate.

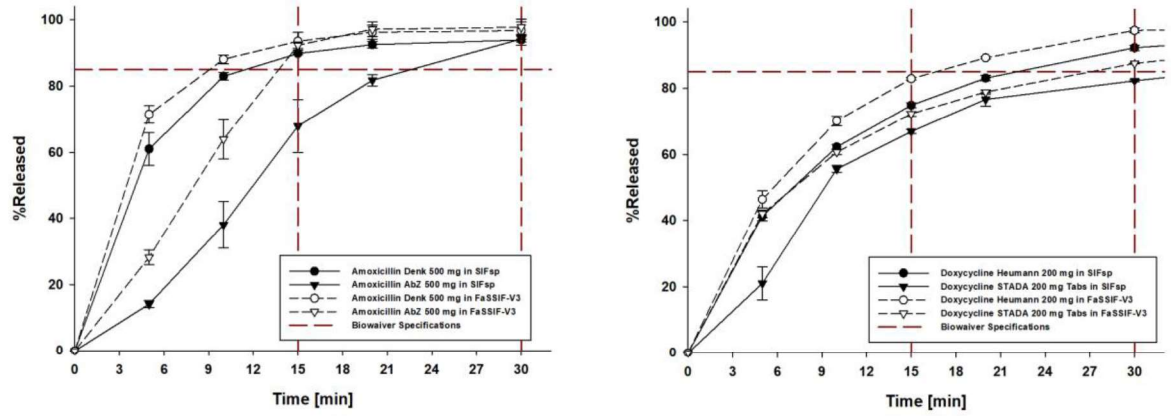

FIGURE 17: *Release profiles of selected SODF containing amoxicillin (**left hand figure**) or doxycycline (**right hand figure**) using Peak Vessels™ in the USP II apparatus and 500 mL of dissolution media at 37°C and 75 RPM. Drug products were tested in compendial (SIF_{sp} pH 6.8) and biorelevant media (FaSSIF-V3). Error bars depict standard deviations (n=3) [reprinted from PUBL. 6 with permission from Elsevier].*

Both the results from the international and national comparison of generic drug products raise questions regarding the suitability of the current regulatory dissolution specifications and experimental setup for a BCS-based biowaiver. Drug products that are interchangeable on the German market showed differences large enough to preclude the application of the BCS-based biowaiver, while some doxycycline monohydrate products with a borderline BCS-class I/II classification, that would, strictly speaking, preclude the application of the BCS-based biowaiver, were able to meet the specifications. The current results, in addition to the many examples in the literature where the regulatory specifications were reported to be over-discriminating for some drug products[28,35,36,231,232], but regarded as not strict enough in other cases[25,26,37], demonstrate the necessity of verifying and validating the dissolution specifications for individual drug products.

To resolve this issue, the potential effect of differences in dosage form performance on pharmacokinetic outcome parameters relevant for bioequivalence (C_{max}, AUC) needs to be evaluated taking into account the interplay between the dosage form, the range of possible physiological conditions (e.g. pH profiles along the GIT, physiological fluid volumes, absorption windows, etc.) and the pharmacokinetic properties of the API.

Thus, to more reliably assess the implications of differences in dosage form performance, the *in vitro* behaviour of the dosage forms was parameterized for input into the *in silico* biopharmaceutical modelling and simulation software GastroPlus®.

4.4. Suitability of Regulatory Dissolution Specifications

4.4.1. Parameterization of *in vitro* Data for Evaluation in *in silico* Models

In addition to other aspects measured *in vitro* (e.g. pH and temperature dependent degradation, pH-solubility profiles, and precipitation studies), data obtained from dissolution experiments had to be parameterized for use in *in silico* models. This was achieved using a combination of the z-factor model as a simplified, mechanistic particle dissolution model and empirical description of the disintegration process utilizing a Weibull-function[233].

The suitability of the z-factor model for simulation of *in vitro* dissolution was investigated in PUBL. 5. The z-factor model can be utilized as an input option for dissolution data in Gastro-Plus® and has been applied in several recent studies[172,234,235], albeit with varying success. Strictly speaking, application of the model is valid only when the complete dose of an API is immediately available for dissolution (i.e. the disintegration time is negligible and coning does not occur). However, the model was utilized in some reported cases where the assumption of immediate availability of the complete dose was clearly not valid, thus likely falsifying the z-factor used to calculate the dissolution rate[234,235]. In order to account for disintegration and the occurrence of coning, modifications in the application of the z-factor model were proposed in PUBL. 5.

FIG. 18A depicts different approaches to fitting the z-factor model to dissolution results obtained from experiments conducted with either regular vessels (where coning occurred and limited the dose available for dissolution) or Peak Vessels™ (where no coning occurred). When the model was fitted to the experimental data from regular vessels, without accounting for the reduced dose available for dissolution, a simulated dissolution profile was obtained (solid blue line) that did not match either of the observed profiles. Only when the highest dose released in the dissolution experiment (obtained from the plateau of the profile) was considered as the mass available for dissolution, was a good representation of the dissolution in regular vessels and, after extrapolation to the complete dose, in Peak Vessels™ achieved (dashed lines).

Similar results were achieved for dissolution profiles obtained with different rotational speeds, as depicted in FIG. 18B. When the reduced dose available for dissolution due to coning at 50 RPM was taken into account, a z-factor (dashed blue line) was obtained that was could accurately describe both the dissolution process at 50 RPM and at 75 RPM (dashed and dotted black lines, respectively), when applying the highest observed amount released as the available dose.

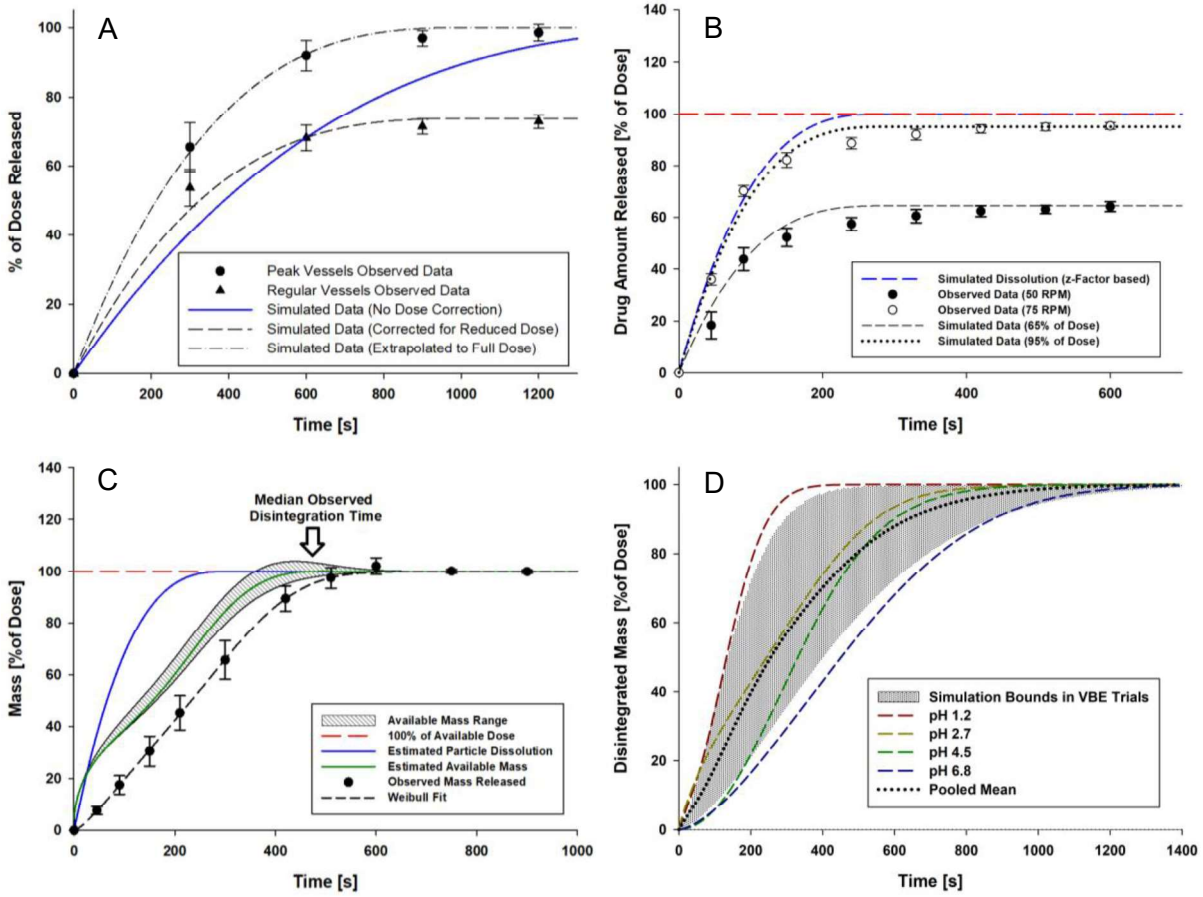

FIGURE 18: *Examples for parameterization of the dosage form performance observed in vitro.* ***(A)*** *Release from a dosage form with negligible disintegration using regular vessels or Peak Vessels™ and z-factor based dissolution simulation accounting for coning.* ***(B)*** *Release from an amoxicillin dosage form with negligible disintegration at 50 and 75 RPM and z-factor based dissolution simulation accounting for coning.* ***(C)*** *Release from a dosage form with significant disintegration time and estimation of the particle dissolution rate and the dosage form disintegration profile.* ***(D)*** *Theoretical disintegration profiles calculated from release profiles at different pH values and simulation bounds used in virtual bioequivalence trials [FIG. 18A-B are reprinted from PUBL. 5, FIG. 18C-D are reprinted from PUBL. 6 and its Supplementary Material with permission from Elsevier].*

In a similar manner, disintegration can limit the mass available for dissolution and thus confound the estimation of the z-factor. To account for the time-dependent change in available mass during disintegration, a numerical approach for calculation of a theoretical disintegration profile described by Nelson and Wang[111] was used. Based on the dissolution factor $\frac{z \cdot C_s}{3}$ and the cumulative fraction of dissolved mass M_n, the change in fraction of disintegrated mass w_i between two time points can be calculated from EQ. 6:

$$\mathrm{M_n} = \sum_{i=0}^{n} w_i \cdot \left\{1 - \left[1 - \frac{z \cdot C_s}{3} \cdot (t_n - t_i)\right]^3\right\} \qquad \text{(EQ. 6)}$$

To interpolate the observed dissolution data in order to obtain a continuous dissolution profile, a Weibull-function was fitted to the mean observed dissolution data. Theoretical disintegration profiles were calculated using time intervals of 1 second. The z-factor used for calculation of the disintegration profile was adjusted so that the resulting theoretical disintegration profile only contained realistic values (i.e. no values above 100% and no decrease in fraction of disintegrated mass between time points) and matched the median, visually observed disintegration time. FIG. 18C exemplarily depicts the approach for the dissolution of DOXYCYCLIN AL 200 T in SIF_{sp}. The observed dissolution profile can be described by a combination of z-factor based dissolution of individual drug particles (solid blue line) and disintegration of the dosage form (solid green line), and was incorporated as such in subsequent GastroPlus® simulations. For each dosage form, z-factor vs. pH profiles were fitted using z-factor estimates obtained at pH 1.2, 2.7, 4.5 and 6.8. To simulate the disintegration of the dosage forms, the range of the calculated disintegration profiles was modelled using Weibull-functions (FIG. 18D).

4.4.2. GastroPlus® Model Setup and Validation

The complete model setup is explained in detail in PUBL. 6. Physicochemical and particular pharmacokinetic parameters were obtained from the literature (LogP, fraction unbound in plasma, apparent permeability), from experimental data (solubility, precipitation time, chemical degradation rates), or were estimated based on the APIs molecular structure (e.g. diffusion coefficients) using the ADMET Predictor® V9.0 (Simulations Plus Inc., Lancaster, USA). Simulating the transit through and absorption from the GIT, a dynamic fluid ACAT-model was chosen, with fluid volumes and gastric emptying times adjusted to values reported in the literature[42,236]. Post-absorptive distribution and elimination were modelled according to 3-compartmental pharmacokinetic models that were fitted to data from clinical trials reported in the literature in which i.v. solutions were administered[225,237]. Parameters fundamental to oral absorption (effective permeability of doxycycline, Michaelis-Menten constants for active transport of amoxicillin via hPEPT) were fitted and verified using literature data from studies in which liquid dosage forms were orally administered to healthy subjects[238,239]. As a last step for validating the models, they were applied to literature data from a set of bioequivalence trials in which solid oral dosage forms were administered[239]. Examples comparing the simulated and observed plasma profile for tablet formulations containing 500 mg amoxicillin and 200 mg doxycycline monohydrate, respectively, are depicted in FIG. 19. The absolute average fold error (AAFE) for all simulations was < 2, a criterion regularly applied in evaluating the success of a model to appropriately describe observed pharmacokinetics[240,241]. Therefore, the established models for the two APIs can be regarded as suitable for describing the absorption process and resulting pharmacokinetics.

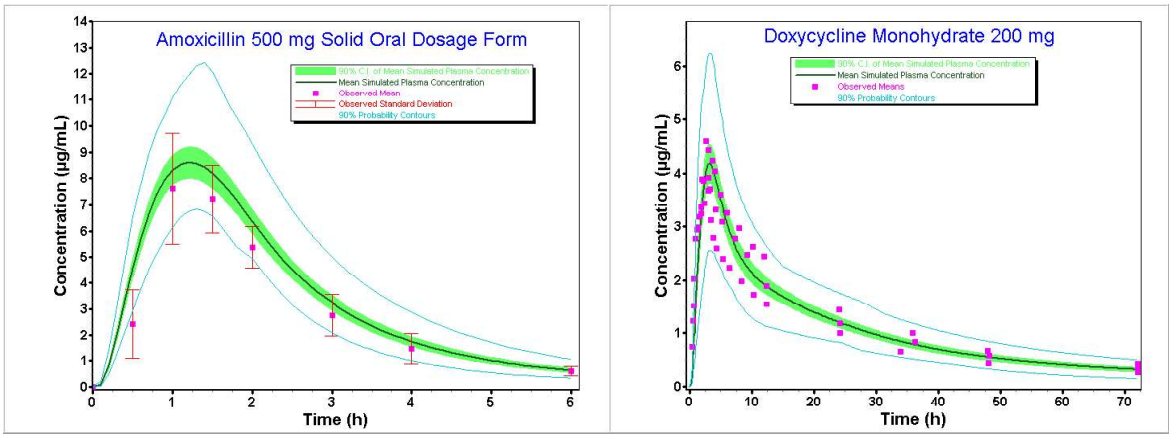

FIGURE 19: *Simulated mean plasma concentrations and 90% probability contours for the administration of 500 mg amoxicillin (**left hand figure**) or doxycycline monohydrate (**right hand figure**) and comparison with data reported in the literature[239][reprinted from Supplementary Material of PUBL. 6 with permission from Elsevier].*

4.4.3. Parameter Sensitivity Analysis (PSA) and Virtual Bioequivalence (VBE)

The established models for amoxicillin and doxycycline were then used to assess the potential impact of differences observed among dosage forms *in vitro* on the *in vivo* pharmacokinetics (PUBL. 6). Data from dissolution experiments with a rotational speed of 50 RPM were used for parameterization of particle dissolution (z-factor based) and dosage form disintegration (empirical description with a Weibull function) of the generic drug products as explained in section 4.4.1. Dissolution data obtained with low rotational speeds were chosen to apply a 'worst case' approach to utilize the broadest possible range of differences in the drug products' dissolution and disintegration behaviour.

For highly soluble drugs (and more specifically BCS class I drugs), C_{max} is expected to be the pharmacokinetic parameter affected the most by variations in dosage form disintegration and particle dissolution[212,242,243]. Thus, this parameter was chosen to compare the performance of the various generic drug products in a PSA. To cover a broad range of physiological scenarios, the influence of variations in gastric pH and emptying rate on simulated C_{max} was investigated.

Results of the PSA are depicted in FIG. 20. The simulated C_{max} values of the designated comparator products (figures in the middle) are compared to the drug products with slowest (left hand figures) and fastest *in vitro* release (right hand figures). Depending on the API and, in the case of doxycycline, also the different salt forms, profiles were obtained that highlight specific physiological factors that can be regarded crucial for a successful comparison in bioequivalence trials.

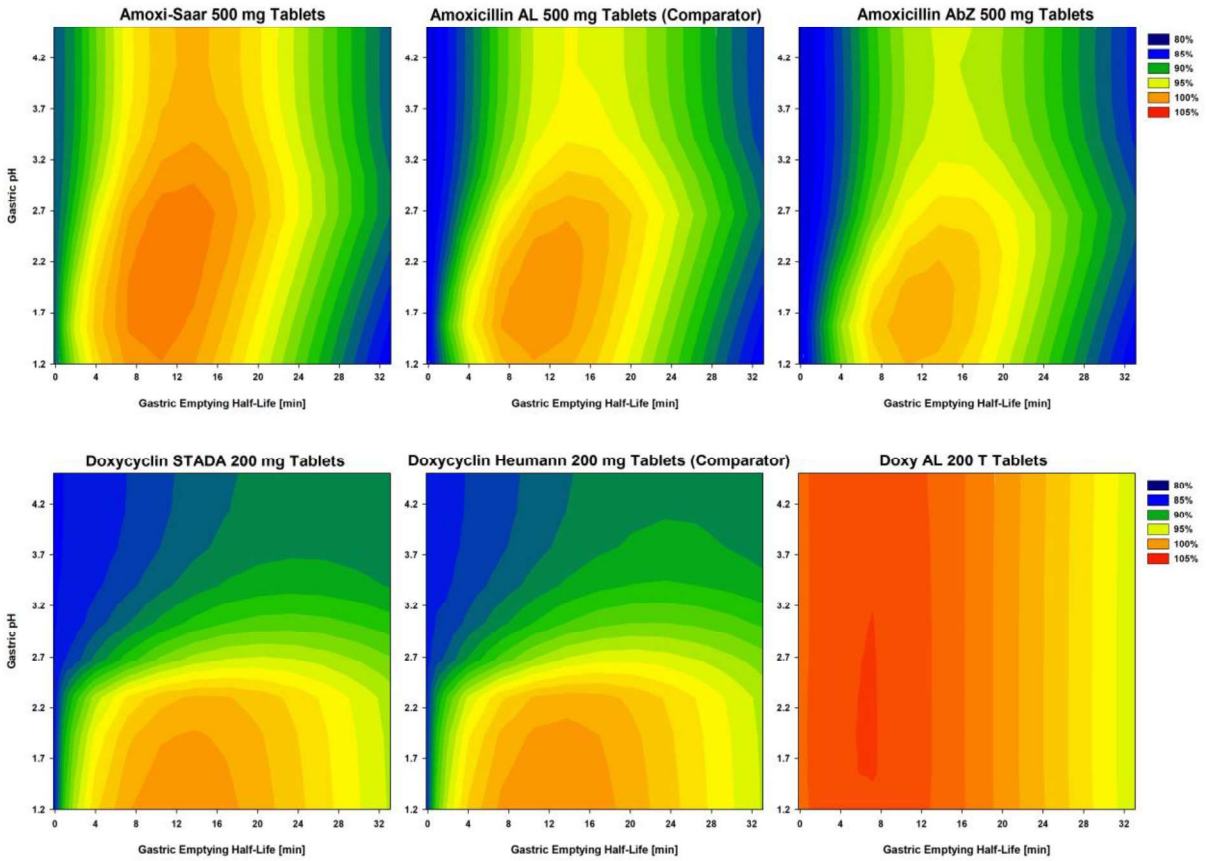

FIGURE 20: *Heat map from parameter sensitivity analysis (PSA) evaluating the impact of changes in gastric emptying rate and gastric pH on C_{max} for selected drug products containing amoxicillin (**top figures**) or doxycycline (**bottom figures**). Coloured areas indicate C_{max} concentrations relative to the highest simulated C_{max} in the PSA of the comparator drug product for the respective API [reprinted from PUBL. 6 with permission from Elsevier].*

All amoxicillin drug products were found to be rather robust to changes in gastric pH. Changes in gastric emptying time, however, noticeably affected the simulated C_{max}, with lower values being observed with either very rapid or protracted gastric emptying. Herein, the lower C_{max} values observed with rapid gastric emptying reflect the presence of an absorption window for active transport of amoxicillin via hPEPT in the proximal small intestine.

The opposite was observed for drug products containing doxycycline monohydrate: the generics were more robust towards changes in gastric emptying rate, but showed a clear dependency of simulated C_{max} on gastric pH, with low values being observed for pH values above 2.5, especially in combination with rapid gastric emptying. This result can be explained with the lower solubility and slower *in vitro* dissolution rate at higher pH values.

For Doxycyclin AL 200 T, the only drug product containing the hyclate salt of doxycycline, no dependency of C_{max} on gastric pH was observed, and gastric emptying rate only had a mild influence on simulated pharmacokinetics, concordant with the higher solubility of the doxycycline hyclate salt in the physiological pH range compared to the monohydrate form.

While the biopharmaceutical properties of the APIs and their salt forms were the major factor determining the distribution profile of C_{max} values in the PSA heat map, the differences observed in *in vitro* dosage form performance only contributed to minor variations among the generics of a specific API. In the case of amoxicillin, overall simulated C_{max} values were slightly lower and the range of gastric emptying rates over which high C_{max} values were simulated was narrower for the slow-disintegrating AMOXICILLIN ABZ compared to the drug product with the fastest release, AMOXI-SAAR.

Consequences with regard to bioequivalence of the drug products that might result from the differences in release rate and disintegration are hypothesized to be unlikely, as all products still achieved high C_{max} values when simulated gastric pH and emptying rate values were within the range that is usually observed in healthy adults (i.e. a gastric pH ≤ 2.7[46] and an average gastric emptying half-life of ~12 min[50]).

This hypothesis was subsequently confirmed in virtual bioequivalence (VBE) trials. A bracketing approach was adopted, whereby the drug product with the slowest overall release was compared to the one with fastest release. Individual virtual subjects were created and their physiological parameters (e.g. subject weight, GI fluid volumes, transit times, pH values, compartmental PK rate constants, effective permeabilities, Michaelis-Menten constants, etc.) and dosage form disintegration time were stochastically generated and distributed within predefined physiological limits. Similar to *in vivo* BE trials, administration of the drug products in the fasted state was simulated using a crossover-design. Pharmacokinetic outcome parameters C_{max} and AUC were used for evaluation of BE between the drug products. Results from five VBE trials for each drug product are depicted in FIG. 21.

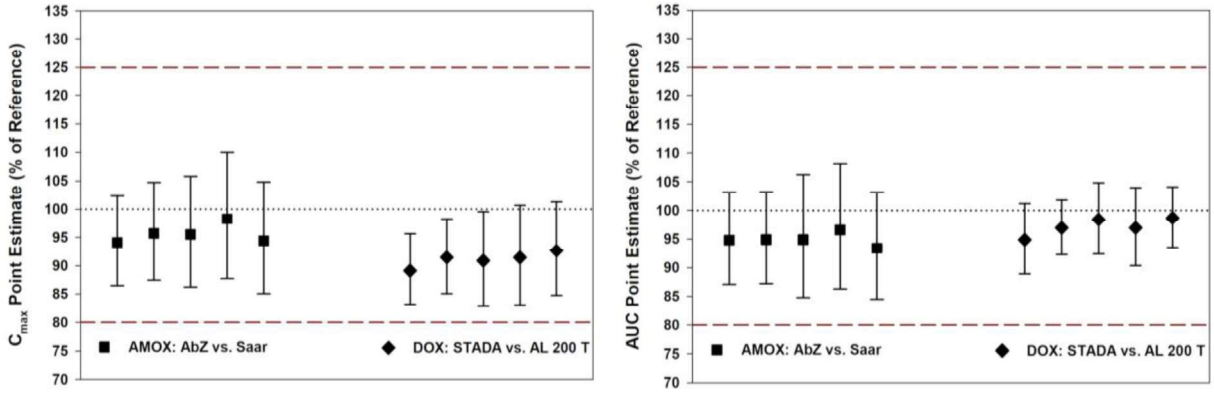

FIGURE 21: *Point estimates for C_{max} (**left hand figure**) and AUC (**right hand figure**) from five virtual bioequivalence trials with n=12 (amoxicillin) and n=18 (doxycycline) virtual subjects, respectively. Drug products with the slowest observed in vitro dissolution and/or disintegration were compared to the respective drug products with the fastest release and/or disintegration. Error bars indicate the 90% confidence intervals. Dashed lines depict specifications for bioequivalence (80.00% - 125.00%) [reprinted from PUBL. 6 with permission from Elsevier].*

Even when the drug products with the fastest release were compared to these with the slowest release, virtual bioequivalence could be demonstrated in the simulated trials for all drug products containing the same API. This finding is in accordance with the market authorization status of the drug products in Germany, as well as with their interchangeable use in the public healthcare sector. This further implies that the regulatory dissolution specifications for a BCS-based biowaiver are overly strict in this case and do not reliably discriminate between bioequivalent and non-bioequivalent drug products.

To further evaluate the suitability of the regulatory "one size fits all" specifications for demonstration of similarity among drug products containing amoxicillin or doxycycline, and to investigate potential modifications that allow for wider limits (e.g. ≥ 85% dissolution in ≤ 40 minutes), VBE trials were conducted with virtual drug products that exemplify a range of potential dissolution scenarios.

4.4.4. Establishing "Safe-Space" Dissolution Specifications for Individual APIs

For verification of the current dissolution specifications and evaluation of potential extensions thereof, virtual drug products were compared to the designated comparator product of each API. For modelling API release from virtual products, z-factors were calculated that yield 85% release in 15, 20, 30 or 40 min, respectively, independent of media pH and under the assumption of sink conditions (usually present in BCS-based biowaiver conform dissolution tests). Results of the VBE trials based on evaluation of simulated C_{max} are depicted in FIG. 22.

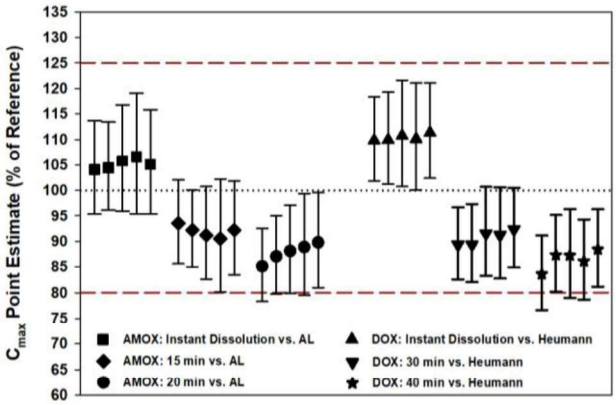

FIGURE 22: *Point estimates for C_{max} of amoxicillin and doxycycline from virtual bioequivalence trials comparing the designated comparator drug product to different in vitro dissolution specifications. Error bars indicate the 90% confidence intervals. Dashed lines depict BE specifications (80.00% – 125.00%) [reprinted from PUBL. 6 with permission from Elsevier].*

Simulated drug products that release ≥ 85% of the API in up to 15 minutes were found bioequivalent to the designated comparator product of amoxicillin, AMOXICILLIN AL. However, when longer dissolution times were used, the 90% CI exceeded the lower specification limit in several cases, indicating a possible risk for non-bioequivalence. Thus, for demonstration of

dissolution similarity of amoxicillin drug products *in vitro*, the regulatory VRD criterion is safe but cannot be extended to include wider specification limits based on the results of this study.

Interestingly, for doxycycline monohydrate drug products, a wider range of dissolution specifications was found to be adequate for ensuring similar *in vivo* performance. Simulated drug products that would release ≥ 85% in 30 minutes under sink conditions *in vitro* were still found to yield virtual bioequivalence with the designated comparator drug product, DOXYCYCLIN HEUMANN. This is in accordance with the regulatory RD criterion, although the results of the VBE trials suggest that when both the doxycycline comparator and test product release their content in ≤ 30 min, additional comparison of the dissolution profiles via f_2-test is not a general requirement for ensuring comparable pharmacokinetic behaviour.

The case examples of amoxicillin and doxycycline demonstrated that the BCS class by itself is not adequate for establishing suitable dissolution specifications, since for amoxicillin, a highly soluble drug, a narrower range of suitable dissolution specifications was observed compared to doxycycline monohydrate, a borderline BCS I/II drug. Only when the interplay between physicochemical, biopharmaceutical and pharmacokinetic parameters was taken into consideration in virtual trials, could a comprehensive assessment of the impact of differences in dissolution performance be made. Application of this approach has demonstrated that, although amoxicillin in a dosage strength of 500 mg has a more favourable BCS class compared to doxycycline monohydrate, differences in dissolution can have a greater impact on its pharmacokinetic parameters. This is because the active uptake of amoxicillin is tied to an absorption window in the proximal small intestine, with the resulting C_{max} further influenced by its short elimination half-life. In contrast, doxycycline exhibits moderate to high permeability across the complete intestinal tract and a long elimination half-life, leading to a later t_{max} and C_{max} being less dependent on differences in dissolution behaviour.

Although confirming the safety of regulatory BCS-based biowaiver criteria, as false-positive outcomes are not expected for complying drug products, the safe-space dissolution criteria derived from the virtual trials could not contribute to a higher proportion of tested generic amoxicillin and doxycycline drug products successfully passing the dissolution comparison. Both the regulatory and the “safe-space” approach were found overly strict for the tested drug products in view of the market authorization of the products tested. Thus, instead of tying the successful comparison of dosage form performance to rigid specifications, differences in dosage form behaviour should rather be assessed in virtual bioequivalence trials that account for a wider range of dissolution scenarios.

Nonetheless, “safe-space” dissolution criteria can still be regarded as a valid approach for verifying the regulatory dissolution specifications or extending the criteria for APIs not investigated in this study, as confirmed by several case examples from the literature[31,35–37,181].

5. Summary and Outlook

The majority of drugs formulated as IR SODF and listed on the 21st WHO EML are highly soluble and thus potential candidates for a BCS-based biowaiver. However, the applicability of the procedure was limited in the past due to a lack of harmonization of regulatory guidances, and practical obstacles, especially for dosage forms with pH-dependent dissolution, a strong influence of coning or a considerable disintegration time, are still preventing its widespread use to the present day.

The ongoing process of harmonizing the BCS-based biowaiver guidelines is a welcome approach facilitating multi-national approval of generics, although strict specifications for demonstrating dissolution similarity still prevent a large fraction of otherwise eligible generic drug products from benefiting of a facilitated approval procedure. This was substantiated based on a retrospective analysis of dissolution results as well as new experimental data for drug products containing amoxicillin or doxycycline.

A potential solution for making the BCS-based biowaiver accessible for more drug products while still maintaining its discriminative power is a shift in the regulatory strategy from the current 'one size fits all' approach to individual specifications. Such specifications are preferably established independently of a drugs BCS class using *in vitro-in silico* approaches and should reflect the complex interplay between physicochemical properties of the API, particularities of the dosage form and the expected *in vivo* behaviour. Parameterization of the *in vitro* dissolution results and subsequent use in validated GastroPlus® models for simulating a variety of scenarios in virtual bioequivalence trials was shown to be a suitable approach to assess the *in vivo* relevance of differences in the dissolution behaviour of generic drug products.

Further contributing to improving the physiological relevance by making the experimental setup more biopredictive and thus more sensitive to formulation differences relevant to the *in vivo* situation, modifications to the experimental setup, such as the use of biorelevant media in addition to setups that help to prevent coning, were demonstrated to be viable tools.

One major limitation of the experiments performed in the framework of this thesis is the use of drug products with proven bioequivalence. In order to establish dissolution specifications that reliably discriminate between *in vivo* bioequivalent and inequivalent drug products, the specifications would have to be further validated using *in vivo* **in**equivalent drug products. However, the availability of such drug products is a limiting factor, as non-BE is rarely observed with BCS class I and III drugs, so that a sufficient number of drug products from failed bioequivalence trails for validation of the approaches presented in this thesis is very difficult to obtain.

A further step based on the approach of simulating the pharmacokinetics of a drug product by combining *in vitro* with *in silico* tools presented in this thesis would be the consideration of pharmacodynamic effects in PK/PD models. Such models could be used for the evaluation of therapeutic equivalence of generic drug products, and have already been suggested for certain APIs such as ibuprofen, where the pharmacokinetic profile of the drug was linked to its efficacy in pain relief[244,245]. However, a lot of data of sufficient quality is needed, e.g. distribution kinetics of the drug from the systemic circulation into the various body tissues, preferably taking into account differences in ADME parameters among patients and healthy subjects. In addition, a valid correlation is needed between the drug concentration in a body tissue and the corresponding efficacy or toxicity.

For the antibiotic agents used as case examples in this thesis, the minimal inhibitory concentrations (MIC) needed to successfully treat infections with various bacterial pathogens could be obtained from publicly available databases such as the EUCAST database (https://www.eucast.org/) and used for establishing a PK/PD model. This would contribute even further to the idea of establishing individual specifications for assessing the therapeutic equivalence of drug products, as all aspects relevant for the therapeutic efficacy of an individual oral drug product are taken into account, starting with the biopharmaceutical behaviour of the dosage form in the gastrointestinal lumen, through distribution of the API from the systemic circulation to various body tissues and finally to the estimation of resulting pharmacodynamic effects.

6. German Summary (Deutsche Zusammenfassung)

Generische Arzneimittel werden von der Europäischen Arzneimittel-Agentur (EMA) als Arzneimittel definiert, die im Vergleich zu einem Referenzarzneimittel hinsichtlich bestimmter Aspekte identisch sind, wie beispielsweise in der Anzahl und Art der Wirkstoffe, der Dosisstärke, dem Ort und der Art der Anwendung, der medizinischen Indikation sowie der einzuhaltenden Qualitätsstandards während der Herstellung. Sie können sich jedoch in ihrem äußeren Erscheinungsbild (Name, Verpackung und Aussehen der Darreichungsform) und vor allem in der qualitativen und quantitativen Zusammensetzung hinsichtlich der bei ihrer Herstellung verwendeten Hilfsstoffe unterscheiden.

Um die therapeutische Äquivalenz im Vergleich zum Referenzarzneimittel und damit auch die Austauschbarkeit im Rahmen der Gesundheitsversorgung der Gesellschaft mit Arzneimitteln sicherzustellen, sind Bioverfügbarkeitsstudien erforderlich. In diesen wird die Bioäquivalenz des Generikums und des Vergleichspräparates nachgewiesen, indem ihr resultierendes pharmakokinetisches Profil hinsichtlich der Geschwindigkeit und des Ausmaßes der Resorption verglichen wird. In Kombination mit der Forderung nach gleichen Qualitätsstandards bei der Herstellung dient der Nachweis der Bioäquivalenz als Beleg für die therapeutische Gleichwertigkeit eines Generikums zum Vergleichspräparat, welche normalerweise in aufwändigeren klinischen Sicherheits- und Wirksamkeitsstudien nachgewiesen werden müsste.

Für Arzneimittel, die als feste, perorale, schnell freisetzende Darreichungsformen formuliert sind, kann der regulatorische Aufwand für die Zulassung generischer Arzneimittel weiter reduziert werden, da auf den Nachweis der Bioäquivalenz *in vivo* zugunsten eines vergleichenden Freisetzungstests *in vitro* im Rahmen eines sogenannten *Biopharmaceutics Classification System (BCS) basierten Biowaivers* für hochlösliche Wirkstoffe verzichtet werden kann. Die Anwendung des BCS-basierten Biowaivers kann in der Folge unnötige Arzneistoffanwendungen am Menschen sowie die Kosten von Bioäquivalenzstudien einsparen und erfasst dabei gleichzeitig einen der wichtigsten Qualitätsaspekte fester Darreichungsformen: Die Arzneistofffreisetzung aus der Darreichungsform und die nachfolgende Auflösung der Wirkstoffpartikel.

Neben potenziellen Einsparungen für den Pharmazeutischen Unternehmer durch die Anwendung eines BCS-basierten Biowaivers kann die vereinfachte Zulassung ebenfalls zu einer breiteren Verfügbarkeit von Generika und dadurch zur Reduzierung eines großen Kostenfaktors im öffentlichen Gesundheitswesen beitragen: In Deutschland wurden 2018 ca. 78% der definierten Arzneimittel-Tagesdosen durch Generika und Biosimilars gedeckt, wobei sie lediglich 23% des Budgets für Arzneimittel und nur 9,3% des Gesamtbudgets im öffentlichen Gesundheitssystem ausmachten.

Im Vergleich zu Deutschland, wo die umfassende Verfügbarkeit von Arzneimitteln mit geprüfter pharmazeutischer Qualität in der Regel sichergestellt ist, hat die weltweite Gesundheitsversorgung noch deutliches Verbesserungspotenzial. Insbesondere in Entwicklungsländern ist der problemlose Zugang zu Arzneimitteln, die von der Weltgesundheitsorganisation (WHO) als unentbehrlich eingestuft werden nur zum Teil möglich. So schätzte die WHO im Jahr 2011, dass mehr als einem Drittel der Weltbevölkerung kein angemessener Zugang zu lebenswichtigen Arzneimitteln möglich sei. Diese Versorgungslücke in Entwicklungsländern wird vor allem auf die hohen Kosten für Arzneimittel, aber auch auf unzureichende finanzielle Mittel und die schwache Infrastruktur der Kontrollbehörden und des öffentlichen Gesundheitssystems zurückgeführt. Hierbei stellt die Verfügbarkeit von generischen Arzneimitteln essentieller Wirkstoffe einen Schlüsselaspekt bei der Senkung der Behandlungskosten einer Vielzahl von Erkrankungen in Entwicklungsländern dar, da ca. 90% der von der WHO als essentiell aufgeführten Wirkstoffe mittlerweile patentfrei sind und somit als kostengünstige Generika verfügbar gemacht werden können.

Zur Bewältigung der Infrastrukturprobleme arbeitet die WHO mit nationalen Behörden zusammen und veröffentlicht Leitfäden, um die Etablierung nationaler Richtlinien zur Arzneimittelversorgung in Entwicklungsländern zu unterstützen. Im Zuge dessen hat die WHO bereits 2006 eine tabellarische Übersicht zur Löslichkeits- und Permeabilitätsklassifizierung essentieller Arzneistoffe erstellt, die kontinuierlich überarbeitet wird, um potenzielle Kandidaten für einen BCS-basierten Biowaiver unter den unentbehrlichen Arzneistoffen zu identifizieren.

Unterstützend zur WHO-Initiative veröffentlicht die Interessengruppe *BCS und Biowaiver* der Internationalen Pharmazeutischen Föderation (FIP) *Biowaiver-Monographien* mit Schwerpunkt auf unentbehrlichen Arzneistoffen, in welchen für die Öffentlichkeit zugänglich alle Daten in einer Nutzen-/Risikobewertung zusammengefasst werden, die für eine mögliche Anwendung eines BCS-basierten Biowaivers relevant sind. Die Biowaiver-Monographien dienen somit als Entscheidungshilfe, ob eine generische Zulassung mittels BCS-basiertem Biowaiver für einzelne Wirkstoffe und deren Arzneimittel aus wissenschaftlicher Sicht zu empfehlen ist.

Der BCS-basierte Biowaiver ist somit ein vielversprechendes Instrument, das Kosteneinsparungen sowie eine Verringerung des regulatorischen Aufwands im Zuge der behördlichen Zulassung von Generika ermöglicht und dazu beitragen kann, die Zugänglichkeit unentbehrlicher Arzneimittel zu erleichtern. Dabei gibt es jedoch auch Hürden, welche die weitläufige Anwendung des Verfahrens verhindern: Unklare Löslichkeits- und Permeabilitätsklassifizierungen von Wirkstoffen, Arzneimittel, welche die Freisetzungskriterien nicht erfüllen

können, sowie Zweifel an der Eignung der regulatorischen Spezifikationen, Freisetzungsunterschiede *in vitro* erfassen zu können, die für das Verhalten *in vivo* relevant sind, sind nur einige Beispiele für Probleme, die es zu lösen gilt.

In dieser Dissertation werden oben genannte Probleme thematisiert, indem eine umfassende Bewertung der Anwendbarkeit und der Einschränkungen des BCS-basierten Biowaivers in seinem aktuellen, von regulatorischen Behörden vorgeschriebenen Ablauf vorgenommen wird. Mögliche Anpassungen des Verfahrens wurden auf der Grundlage experimenteller *in vitro* Daten untersucht, bewertet und mithilfe von *in silico* Simulationsmodellen auf die Situation *in vivo* extrapoliert.

Der Großteil (~52%) der in sofort freisetzenden, festen, peroralen Darreichungsformen formulierten Arzneistoffe, die auf der aktuellen *Essential Medicines List* geführt sind, konnten in dieser Arbeit als hochlöslich klassifiziert werden und stellen daher potenzielle Kandidaten für einen BCS-basierten Biowaiver dar. Die tatsächliche Anwendung des Verfahrens war jedoch in der Vergangenheit aufgrund mangelnder Harmonisierung der behördlichen Richtlinien eingeschränkt, und noch immer verhindern praktische Hindernisse dessen Durchführung, insbesondere für Darreichungsformen mit pH-Wert-abhängiger Auflösung oder einer beträchtlichen Zerfallszeit.

Der fortlaufende Prozess der Harmonisierung der BCS-basierten Biowaiver-Richtlinien ist ein willkommener Ansatz, der die multinationale Zulassung von Generika erleichtert, obwohl strenge Spezifikationen für den Nachweis der Ähnlichkeit von Freisetzungsprofilen noch immer verhindern, dass ein großer Teil der ansonsten in Frage kommenden Generika von diesem erleichterten Zulassungsverfahren profitiert. Anhand einer retrospektiven Analyse von Freisetzungsergebnissen der Goethe Universität sowie auf der Grundlage neuer experimenteller Daten für Arzneimittel, die Amoxicillin oder Doxycyclin enthalten, wurde gezeigt, dass ca. 30% der theoretisch für den BCS-basierten Biowaiver geeigneten Arzneimittel nicht die *in vitro* Freisetzungsspezifikationen erfüllen können und zum Teil beträchtliche Unterschiede im biopharmazeutischen Verhalten zwischen einzelnen Generika bestehen.

Eine mögliche Lösung, die in dieser Arbeit vorgestellt wird, um den BCS-basierten Biowaiver dennoch für mehr Arzneimittel zugänglich zu machen und gleichzeitig eine sichere Unterscheidung zwischen potenziell bio**in**äquivalenten Produkten zu gewährleisten, ist eine Abkehr vom derzeitig regulatorisch vorgegebenen „*one size fits all*“ Ansatz hin zu arzneistoffindividuellen Freisetzungsspezifikationen. Diese sollten vorzugsweise unabhängig von der BCS-Klassifizierung und unter Verwendung von *in vitro / in silico*-Methoden festgelegt werden, die das komplexe Zusammenspiel zwischen den physikochemischen Eigenschaften des Arzneistoffes, den Besonderheiten der Darreichungsform und den physiologischen Gegebenheiten *in vivo* widerspiegeln. Die in dieser Arbeit angewandte Parametrisierung der *in vitro*

Freisetzungsprofile zur Unterscheidung von Partikelauflösung und Tablettenzerfall sowie deren anschließende Verwendung in validierten GastroPlus®-Modellen zur Simulation einer Vielzahl physiologischer Szenarien in virtuellen Bioäquivalenzstudien erwies sich als geeigneter Ansatz zur Bewertung der *in vivo* Relevanz von *in vitro* Unterschieden im Auflösungsverhalten von Generika.

Weiterhin konnte gezeigt werden, dass die Verwendung von biorelevanten Medien zusätzlich zu einem instrumentellen Aufbau unter Verwendung von *Peak Vessels™*, die das Entstehen hydrodynamisch „toter Zonen" verhindern, geeignete Maßnahmen sind, um den Versuchsaufbau bioprädiktiver und empfindlicher für Formulierungsunterschiede zu machen, die für die Situation *in vivo* relevant sind.

Eine wesentliche Limitierung der im Rahmen dieser Arbeit durchgeführten Experimente ist jedoch die Verwendung von Arzneimitteln mit bereits vorausgesetzter Bioäquivalenz. Um Auflösungsspezifikationen festlegen zu können, die zuverlässig zwischen *in vivo* bioäquivalenten und -**in**äquivalenten Arzneimitteln unterscheiden, müssen die Spezifikationen ferner unter Verwendung von *in vivo* als bio**in**äquivalent geprüften Arzneimitteln validiert werden. Die Verfügbarkeit solcher Arzneimittel stellt hierbei jedoch den limitierenden Faktor dar, da bei Arzneimitteln der BCS-Klassen I und III nur äußerst selten Bio**in**äquivalenz *in vivo* festgestellt wird und daher eine ausreichende Anzahl von Arzneimitteln aus fehlgeschlagenen Bioäquivalenzstudien zur Validierung der in dieser Arbeit vorgestellten Ansätze kaum zu erhalten sein wird.

Eine zukünftige Erweiterung des Verfahrens, die auf dem hier vorgestellten Ansatz der Simulation der Pharmakokinetik eines Arzneimittels durch Kopplung von *in vitro* mit *in silico* Methoden basiert, ist die Berücksichtigung pharmakodynamischer Effekte in sogenannten PK/PD-Modellen. Diese Modelle können zur genaueren Bewertung der therapeutischen Äquivalenz von Generika verwendet werden und wurden bereits für einzelne Wirkstoffe wie beispielsweise für Ibuprofen vorgeschlagen, bei welchem das pharmakokinetische Profil des Arzneistoffes mit seiner Wirksamkeit bei der Schmerzlinderung korreliert werden konnte. Für einen solchen Ansatz wird jedoch eine Vielzahl klinischer Daten von hinreichender Qualität benötigt, um unter anderem die Verteilungskinetik des Arzneimittels zwischen dem systemischen Kreislauf und verschiedenen Körpergeweben exakt beschreiben zu können, vorzugsweise unter Berücksichtigung von Unterschieden in ADME-Parametern zwischen Patienten und gesunden Probanden. In jedem Fall ist eine valide Korrelation zwischen der Wirkstoffkonzentration in einem Körpergewebe und der daraus resultierenden pharmakodynamischen Effekte zwingend erforderlich.

Für die in dieser Arbeit als Fallbeispiele verwendeten Antibiotika Amoxicillin und Doxycyclin können die zur therapeutischen Infektionsbehandlung erforderlichen minimalen Hemmkonzentrationen für verschiedene Erreger aus öffentlich zugänglichen Datenbanken wie der EUCAST-Datenbank bezogen und zur Erstellung eines PK/PD-Modells verwendet werden. Dies würde noch weiter zu dem Leitgedanken beitragen, individuelle Spezifikationen zur Bewertung der therapeutischen Äquivalenz von Arzneimitteln festzulegen, da somit alle für die therapeutische Wirksamkeit eines bestimmten, peroral angewendeten Arzneimittels relevanten Aspekte berücksichtigt würden, beginnend mit dem biopharmazeutischen Verhalten der Darreichungsform im Magen-Darm-Lumen, über die Verteilung der Wirkstoffes aus dem systemischen Kreislauf in verschiedene Körpergewebe bis zu den daraus resultierenden pharmakodynamischen Effekten.

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Appendix

A.1. Publications

A.1.1. Publication list

Publications in peer-reviewed journals:

1. Plöger GF, Hofsäss MA, Dressman JB. Solubility determination of active pharmaceutical ingredients which have been recently added to the list of essential medicines in the context of the biopharmaceutics classification system-biowaiver. J Pharm Sci. 2018;107(6):1478-1488. (as *equal first author*)
2. Hofsäss MA et al. Biowaiver monographs for immediate-release solid oral dosage forms: folic acid. J Pharm Sci. 2017;106(12):3421-3430.
3. Hofsäss MA and Dressman J. The discriminatory power of the BCS-based biowaiver: a retrospective with focus on essential medicines. J Pharm Sci. 2019;108(9):2824-2837.
4. Lehmann A, Hofsäss M, Dressman J. Differences in drug quality between South Africa and Germany. J Pharm Pharmacol. 2018;70(10):1301-1314. (as *equal first author*)
5. Hofsäss MA and Dressman J. Suitability of the z-factor for dissolution simulation of solid oral dosage forms: potential pitfalls and refinements. J Pharm Sci. 2020;109(9):2735-2745.
6. Hofsäss MA and Dressman J. Evaluation of differences in dosage form performance of generics using BCS-based biowaiver specifications and biopharmaceutical modelling – case examples amoxicillin and doxycycline. J Pharm Sci. 2020;109(8):2437-2453.

Poster presentations at scientific conferences:

1. Hofsäss MA, Born GF, Dressman JB. Establishing the BCS classification of APIs recently added to the WHO essential medicines list. AAPS Annual Meeting, Orlando, USA, 2015
2. Hofsäss MA and Dressman JB. Investigation of the discriminatory power of dissolution specifications for waiving *in vivo* bioequivalence via pharmacokinetic simulation – case example doxycycline. 11th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Granada, Spain, 2018.
3. Hofsäss MA and Dressman JB. Investigation of the discriminatory power of dissolution specifications for BCS-based biowaiver via pharmacokinetic modelling – case example amoxicillin. 3rd European Conference on Pharmaceutics, Bologna, Italy, 2019.

A.1.2. Personal contribution to the peer-reviewed publications

1. Plöger G, Hofsäss MA, Dressman JB. Solubility determination of active pharmaceutical ingredients which have been recently added to the list of essential medicines in the context of the biopharmaceutics classification system-biowaiver. J Pharm Sci. 2018;107(6):1478-1488. (as *equal first author*)

Author contribution (Martin Hofsäss):

Contributed to literature research (in particular: permeability data for investigated active pharmaceutical ingredients), conducted the solubility assays of the active pharmaceutical ingredients amiodarone, atazanavir, enalapril, folic acid, hydroxychloroquine, mifepristone, oseltamivir, ribavirin and rifabutine, contributed to data processing, evaluation and interpretation, contributed to drafting and editing the manuscript (in particular the sections “results” and “discussion”).

Author contribution of cooperation partner (Gerlinde Plöger):

Contributed to literature research, conducted the solubility assays of the active pharmaceutical ingredients cyclizine, dexamethasone, emtricitabine, medroxy-progesterone, mesna, morphine and succimer. contributed to data processing, evaluation and interpretation, contributed to drafting and editing the manuscript (in particular the sections “introduction” and “materials and methods”).

Author contribution of cooperation partner (Jennifer Dressman):

Supervised the study as part of the Collaborating Centre of the WHO, edited the manuscript draft.

2. Hofsäss MA, Souza J, Silva-Barcellos NM, Bellavinha KR, Abrahamsson B, Cristofoletti R, Groot DW, Parr A, Langguth P, Polli JE, Shah VP, Tajiri T, Mehta MU, Dressman JB. Biowaiver monographs for immediate-release solid oral dosage forms: folic acid. J Pharm Sci. 2017;106(12):3421-3430.

Author contribution (Martin Hofsäss):

Performed literature research, conducted the solubility assay of the active pharmaceutical ingredient folic acid, contributed to summarizing, evaluation and interpretation of literature search results, contributed to drafting of the manuscript, devised all figures and table 2 (except table 3), revised and edited all sections of the manuscript.

Author contribution of cooperation partners (Jacqueline de Souza, Neila Silva-Barcellos, Karime Bellavinha):

Performed literature research, devised table 2, contributed to summarizing, evaluation and interpretation of literature search results, contributed to drafting the manuscript.

Author contribution of cooperation partners (Bertil Abrahamsson, Rodrigo Cristofoletti, Alan Parr, Peter Langguth, James E. Polli, Vinod P. Shah, Tomokazu Tajiri, Mehul U. Mehta):

Commented on the manuscript draft for revisions.

Author contribution of cooperation partner (D.W. Groot):

Summarized and drafted excipient list (table 3), commented on the manuscript draft for revisions.

Author contribution of cooperation partner (Jennifer Dressman):

Devised the monograph concept, edited the manuscript draft.

3. Hofsäss MA and Dressman JB. The discriminatory power of the BCS-based biowaiver: a retrospective with focus on essential medicines. J Pharm Sci; 2019;108(9):2824-2837.

Author contribution (Martin Hofsäss):

Jointly devised the conceptual idea, performed literature research and retrospective data analysis of dissolution results, performed statistical analysis, evaluation and interpretation of the results, drafted and edited the manuscript.

Author contribution of cooperation partner (Jennifer Dressman):

Jointly devised the conceptual idea, advised in interpretation of the results, edited the manuscript draft.

4. Lehmann A, Hofsäss M, Dressman J. Differences in drug quality between South Africa and Germany. J Pharm Pharmacol. 2018;70(10):1301-1314. (as *equal first author*)

Author contribution (Martin Hofsäss):

Contributed to devising the conceptual idea, contributed to literature research (in particular: skin lightening products), carried out chemical analysis of skin lightening products, performed comparative dissolution testing, contributed to validation of HPLC methods, contributed to interpreting the results, contributed to drafting and editing the manuscript.

Author contribution of cooperation partner (Andreas Lehmann):

Contributed to devising the conceptual idea, contributed to literature research (in particular: public health and regulatory aspects in South Africa), carried out chemical analysis of prescription medicines, contributed to validation of HPLC methods, contributed to interpreting the results, contributed to drafting and editing the manuscript.

Author contribution of cooperation partner (Jennifer Dressman):

Supervision of the studies and editing of the manuscript draft.

5. Hofsäss MA and Dressman JB. Suitability of the z-factor for dissolution simulation of solid oral dosage forms: potential pitfalls and refinements. J Pharm Sci. 2020 (Epub ahead of print, doi:10.1016/j.xphs.2020.05.019).

Author contribution (Martin Hofsäss):

Devised the conceptual idea, performed literature search on studies utilizing the z-factor dissolution model, devised the theoretical background, performed experimental dissolution tests and parameterization of the results, carried out dissolution simulations based on experimental and theoretical examples, evaluated and interpreted the results, drafted and edited the manuscript.

Author contribution of cooperation partner (Jennifer Dressman):

Contributed to the conceptual idea, contributed in discussing the theoretical framework, advised in preparation of the manuscript, edited the manuscript draft.

6. Hofsäss MA and Dressman J. Evaluation of differences in dosage form performance of generics using BCS-based biowaiver specifications and biopharmaceutical modelling – case examples amoxicillin and doxycycline. J Pharm Sci. 2020 (accepted for publication, doi: 10.1016/j.xphs.2020.04.011, Epub ahead of print).

Author contribution (Martin Hofsäss):

Jointly devised the conceptual idea, performed literature search, carried out all experiments and parameterization of the results, established and validated physiologically based biopharmaceutical models for doxycycline and amoxicillin, carried out the virtual parameter sensitivity analysis and virtual bioequivalence trials based, evaluated and interpreted the results, drafted and edited the manuscript.

Author contribution of cooperation partner (Jennifer Dressman):

Jointly devised the conceptual ideal, contributed in the discussion of results and their interpretation, advised in preparation of the manuscript, edited the manuscript draft.

A.1.3. Peer-reviewed publications

A.1.3.1 Publication 1

Solubility determination of active pharmaceutical ingredients which have been recently added to the list of essential medicines in the context of the biopharmaceutics classification system-biowaiver

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Global Health Commentary

Solubility Determination of Active Pharmaceutical Ingredients Which Have Been Recently Added to the List of Essential Medicines in the Context of the Biopharmaceutics Classification System—Biowaiver

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ABSTRACT

Since the publication of Lindenberg et al., which classified orally administered active pharmaceutical ingredients (APIs) on the 2004 Essential Medicines List (EML) of the World Health Organization according to the Biopharmaceutics Classification System (BCS), various APIs have been added to the EML. In this work, BCS classifications for 16 of the orally administered APIs which were added to the EML after 2004 were determined. To establish a reliable solubility classification for all these compounds, a miniaturized shake-flask method was introduced. This method enables a fast, economical determination of the BCS solubility class while reliably discriminating between "highly soluble" and "not highly soluble" compounds. Nine of the 16 APIs investigated were classified as "highly soluble" compounds, making them potential candidates for an approval of multisource drug products via the BCS-based biowaiver procedure. The choice of dose definition (which currently varies among the guidances pertaining to BCS-based bioequivalence published by various regulatory authorities) had no effect on the solubility classification of any of the 16 substances evaluated. BCS classification of the compounds was then completed using permeability data obtained from the literature. As several APIs decomposed at one or more pH values, a decision tree for determining their solubility was established.

Introduction

The World Health Organization (WHO), which was founded in 1948, is part of the United Nations.[1] Generally regarded as the leading authority on international health, its objective is the achievement of the highest possible level of health for all people.[2] According to its constitution, health "is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."[2] As part of its work for global health, the WHO publishes the WHO Model List of Essential Medicines (EML), which includes medicines that are considered indispensable for a well-functioning health system and which should therefore be made available in dosage forms with assured quality at an affordable price.[3] The first version of the EML was released in 1977 and has since then been updated in regular intervals. The current edition is the 20th Essential Medicines List.[4]

The classification of Active Pharmaceutical Ingredients (APIs) listed on the EML based on the Biopharmaceutics Classification System (BCS)[5] is an essential step in determining whether a multisource product is eligible for approval via a BCS-based biowaiver. This procedure eliminates the need for *in vivo* testing of multisource drug products, and thus reduces development costs and time to approval.

According to the BCS, an API can be assigned to 1 of 4 classes based on its solubility and permeability (Fig. 1). Besides requiring that the API belongs to an eligible BCS class, consideration must be given to therapeutic index, stability of the API under gastrointestinal conditions, eligibility of the dosage form for this procedure, and excipient effects on absorption from the gastrointestinal tract. The risks associated with an incorrect positive decision with respect to bioequivalence (BE) (i.e., the dosage form is deemed to be bioequivalent by the BCS-biowaiver procedure but is actually not bioequivalent) are evaluated. As a last step, the *in vitro* dissolution of the generic product is compared with that of the reference product. Various health authorities such as the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), and

Abbreviations used: WHO, World Health Organization; EML, List of Essential Medicines; APIs, Active Pharmaceutical Ingredients; BCS, Biopharmaceutics Classification System; FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; BE, bioequivalence; IR, immediate-release; D/S, dose/solubility; HPLC, high-pressure liquid chromatography; UV, ultraviolet; BA, bioavailability.

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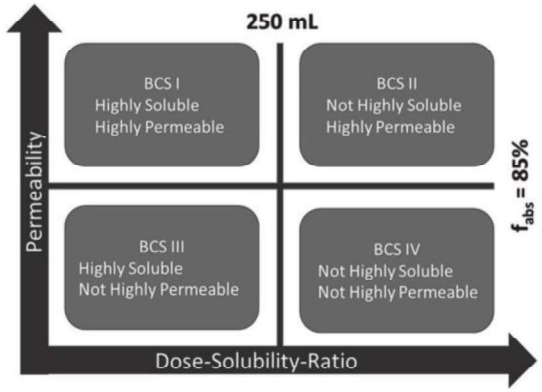

Figure 1. Biopharmaceutics Classification System (modified from Amidon et al.[5]).

the WHO require similar (but not yet fully harmonized) criteria to be fulfilled to grant a biowaiver approval.[6-8] In summary, the BCS-biowaiver procedure is a time- and cost-saving approach for the approval of generic drug products because it is not based on *in vivo* BE studies but on *in vitro* dissolution studies and thus facilitates the realization of WHO's goal to achieve availability of high-quality multisource drug products containing APIs listed on the EML at affordable prices.

In 2004, Lindenberg et al.[9] classified orally administered APIs of the 12th edition of the Essential Medicines List[10] according to the BCS. The classifications were based on solubility and permeability data obtained from the open pharmaceutical literature. Depending on the quality of the data, the APIs were assigned to those with a reliable or a provisional BCS class. Alternatively, it was concluded that the data available were insufficient to reach a conclusion about the BCS class. Experimental solubility data were not obtained in that study because of the large number of APIs under investigation and lack of resources available to experimentally determine the solubility of each API using the standard shake-flask technique.

In 2005, a modification of the shake-flask method was published by Glomme et al.[11] These researchers compared a miniaturized, scaled-down approach with the conventional shake-flask method and showed that the scaled-down method was a cost-effective alternative to the conventional, large-scale approach used in pharmaceutical development. For this reason, scaled-down approaches have become increasingly popular and have been implemented more frequently in the ensuing years.

Combining the scaled-down approach to solubility determination with the need to provide reliable BCS classifications for orally administered APIs that have been added to the EML, the purpose of this study was to experimentally determine the solubility classification of 16 APIs that have been added since the 14th version of the EML.[12] All APIs included in this study are formulated in solid, immediate-release (IR) oral dosage forms and have not previously been reliable classified according to the BCS. Since literature data on the solubility of APIs under BCS-relevant conditions are sparse, and since the definition of the "dose" used for calculating the dose/solubility (D/S) ratio varies between different guidance documents,[6-8] the experimentally determined solubility values of the respective APIs under BCS-relevant conditions are presented in this study. This allows for the calculation of a solubility classification according to the various dose definitions applied across the different jurisdictions. It also enables the BCS classification to be checked in the case where the dosage strength is revised in a future EML version, if the dosage strength is different in a given jurisdiction to the dose recommended by the EML, or if a new dosage strength of the API is added to the products already available.

Materials and Methods

Materials

The 16 APIs included in this study were amiodarone hydrochloride, atazanavir sulfate, cyclizine, dexamethasone, emtricitabine, enalapril maleate, folic acid, hydroxychloroquine sulfate, medroxyprogesterone acetate, mesna, mifepristone, morphine sulfate, oseltamivir phosphate, ribavirin, rifabutin, and succimer.

Folic acid and medroxyprogesterone acetate were already listed on the 12th WHO EML[10] but were also included in this study because of conflicting solubility data in the literature. Dexamethasone was also listed on the 12th EML[10] with a dose strength of 0.5 mg and at that time had been classified as "highly soluble" by Lindenberg et al.[9] It was then withdrawn from the list until the 17th version,[13] when it was listed again, but at a higher dosage strength of 4 mg and for a different indication (Table 1). It was therefore necessary to confirm the "highly soluble" criterion at the higher dose strength of 4 mg. Because the solubility classification of morphine sulfate as "highly soluble" by Lindenberg et al.[9] was based solely on determinations above pH 5.5 at 35°C,[14] it was necessary to perform further studies for this API to obtain a reliable classification over the whole physiological pH range of 1.2-6.8 at 37°C.

Mifepristone was added in the 14th EML, emtricitabine, and ribavirin in the 15th and amiodarone hydrochloride, atazanavir sulfate, mesna, oseltamivir sulfate, and rifabutin in the 16th edition of the WHO EML (Table 1).[12,15,16] Cyclizine, enalapril maleate, hydroxychloroquine sulfate, and succimer appeared as APIs in solid oral dosage forms for the first time on the 17th WHO Essential Medicines List[13] in March 2011.

All APIs were purchased from suppliers in Germany. Information regarding analytical grade, batch numbers, and details concerning retailer and manufacturer can be found in Table 2. All other chemicals used in the studies were of analytical grade. Dipotassium monohydrogenphosphate, disodium monohydrogenphosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, and sodium hydroxide were obtained from VWR® Prolabo® (Leuven, Belgium). All acids and sodium hydroxide (1 M) were purchased from VWR® Prolabo® (Fontenay-Sous-Bois, France). Ammonium acetate, sodium acetate trihydrate, acetonitrile and methanol were obtained from Merck KGaA (Darmstadt, Germany). Ethanol was purchased from AHK Alkoholhandel GmbH & Co. (Ludwigshafen, Germany). Uniprep™ syringeless filters by Whatman™ (Little Chalfont, UK) were used as small-scale filter systems.

Solubility Experiments

The solubility was determined according to the requirements set in the Annex 7 of the WHO technical report series titled "Multisource (generic) pharmaceutical products: guidelines on registration requirements to establish interchangeability," which states that an API is considered "highly soluble" when the D/S ratio is ≤250 mL over the pH range of 1.2-6.8 at 37 ± 1°C.[8] The solubility of all substances and the resulting BCS solubility classification was determined according to the study protocol shown in Table 3.

The solubility studies were based on the shake-flask method, which is used to determine the equilibrium solubility of a substance. In this method, an excess of substance is added to a medium with a certain pH-value, creating a suspension (media compositions are listed in Table 4). The suspension is then shaken for a

Table 1
APIs Examined in the Solubility Study, Along With the Year of First Appearance on the EML, Highest Dose Strength and Drug Class Listed on the 20th EML

Drug	First Listed on EML	Dose Strength on 20th EML (mg)	Drug Class Listed in 20th EML
Amiodarone hydrochloride[C]	16 (2010)	400	Antiarrhythmic
Atazanavir sulfate	16 (2010)	300	Protease inhibitor
Cyclizine	17 (2011)	50	Symptom relief in palliative care
Dexamethasone	17 (2011)	4	Antiemetic
Emtricitabine	15 (2007)	200	Protease inhibitor
Enalapril maleate	17 (2011)	5	Antihypertensive
Folic acid	Before 12 (2002)	5	Antianemia
Hydroxychloroquine sulfate[C]	17 (2011)	200	DMARDs
Medroxyprogesterone acetate	12 (2002)	5	Progestogen
Mesna[C]	16 (2010)	600	Cytotoxics and adjuvants
Mifepristone[C]	14 (2005)	200	Oxytocics
Morphine sulfate	Before 12 (2002)	10	Opioid analgesics
Oseltamivir phosphate	16 (2010)	75	Antivirals
Ribavirin	15 (2007)	600	Antivirals
Rifabutin	16 (2010)	150	Antituberculosis
Succimer	17 (2011)	100	Specific antidotes

[C] Included on the complementary list but not included on the main list.

specified time at a defined temperature to produce an equilibrium between the saturated solution and undissolved solid, that is undissolved substance should still be visible at the end of the shaking period. After a final pH measurement to check whether the pH remained unchanged, the sample is filtered and quantified. The shake-flask method can also be conducted in a miniaturized approach with a reduction in both the amount of drug and volume of medium needed, as previously mentioned.[11] Instead of a flask, a Whatman™ Uniprep™ vial with a 3-mL chamber and a plunger with an integrated polytetrafluorethylene filtration membrane (pore size: 0.45 μm) was used for our experiments.

For highly soluble APIs, the approach was further modified. Instead of determining the thermodynamic (equilibrium) solubility as described above, the "minimum solubility" was determined as follows. According to the criteria of the BCS, a drug can be classified as highly soluble if the D/S ratio is equal to or less than 250 mL.[5] In our solubility studies, the highest dose strength listed on the 20th EML[4] was used as the dose for calculating the D/S ratio for each API. To scale down the experiment, the amount of API that would need to go into solution to correspond to a classification as "highly soluble" if completely dissolved in 3 mL of buffer solution was calculated. An amount at least 50% greater than this calculated amount was accurately weighed into the Uniprep™ vials in triplicate. Three milliliters of the appropriate buffer solution was then added to each Uniprep™ vial. A plunger with an integrated polytetrafluorethylene filter system was mounted on each vial, and the unit was closed. All samples were then shaken on an orbital shaker (Heidolph Polymax 1040) for 24 h at a rotational speed of 45 rpm and a temperature of 37 ± 0.5°C. After 24 h, the vials were visually examined for any excess API solid, and the samples were filtered by pushing the plunger into the Uniprep™ vial. Afterward, an aliquot of the sample was withdrawn from the filtrate and diluted with an appropriate

Table 2
Chemical Reference Standards Used for Solubility Determinations

Drug	Analytical Grade/Purity	Batch	Supplier	Source
Amiodarone hydrochloride	99.8%	P500164	Sigma–Aldrich, Germany	RT-Corp, Laramie, WY, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Atazanavir sulfate	100%	Pure API was obtained from Bristol-Myers Squibb	Bristol-Myers Squibb Company, New Brunswick, NJ, USA	Bristol-Myers Squibb Company, New Brunswick, NJ, USA
Cyclizine hydrochloride	USP Reference Standard	H0D321	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Dexamethasone	European Pharmacopeia (Ph. Eur.) 7.0	13352310	Caelo, Germany	Caesar and Lorentz GmbH, Hilden. Germany
Emtricitabine	USP Reference Standard	F0J163	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Enalapril maleate	100.4%	E13Z017	VWR, Germany	Alfa Aesar, Karlsruhe, Germany
Folic acid	100.2%	K45899584537	VWR, Germany	Merck KGaA, Darmstadt, Germany
Hydroxychloroquine sulfate	USP Reference Standard	K0G211	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Medroxyprogesterone 17-acetate	Ph. Eur. Reference Standard	Ph.Eur. CRS # 3.0 Id: 00ESX7	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Mesna	USP Reference Standard	F0H331	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Mifepristone	100%	SLBJ7154V	Sigma–Aldrich, Germany	Sigma–Aldrich, Co, St. Louis, MO, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Morphine sulfate pentahydrate	Analytical grade (>98%)	SLBL1738V	Sigma–Aldrich, Germany	Sigma–Aldrich Chemie GmbH, Steinheim, Germany
Oseltamivir phosphate	USP Reference Standard	R00490	Sigma–Aldrich, Germany	USP, Rockville, MD, USA
Ribavirin	Ph. Eur. Reference Standard (99.9%)	Ph.Eur. CRS # 2.0 # 2583198	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Rifabutin	Ph. Eur. Reference Standard (95.5%)	Ph.Eur. CRS # 2.0 Id: 0030C1	Sigma–Aldrich, Germany	Council of Europe, EDQM MS, Strasbourg, France
Succimer = meso-2, 3-Dimercaptosuccinic acid	Analytical grade (~98%)	SLBH6371V	Sigma–Aldrich, Germany	Sigma–Aldrich, Co, St. Louis, MO, USA/Sigma–Aldrich Chemie GmbH, Steinheim, Germany

CRS, chemical reference standards; USP, United States Pharmacopoeia.

Table 3
Study Protocol for Solubility Determination of APIs on the EML

Conditions	Comments
1. Preparation of solubility samples in Uniprep™ syringeless filters	An excess of the API was weighed into Uniprep™ vials in triplicate ($n = 3$ for each buffer). Three milliliters of the buffer solution was added to each Uniprep™ vial.[a] All vials were provisionally sealed with the Uniprep™ plunger.
2. Shaking and incubation	Samples were shaken on an orbital shaker at 45 rpm. Temperature during incubation was maintained at 37 ± 0.5°C. Samples were incubated and shaken for 24 h.
3. Filtration	Status of dissolution, that is, whether any solid could be visually detected, was checked before filtration. The Uniprep™ plunger was pushed into the vial to effect filtration.
4. Sampling and dilution	An aliquot of the filtrate was withdrawn and diluted to an appropriate concentration for analysis (determined in preliminary studies).
5. pH measurement	Any changes to the pH value during the dissolution process were evaluated by a final pH measurement.
6. HPLC analysis	The concentration of dissolved drug was quantified via validated HPLC methods using UV detection (see Table 5). Mean solubility values were calculated.
7. Solubility classification based on the BCS	The highest dose strength listed on the 20th EML was divided by the experimentally obtained solubility values to calculate the dose/solubility ratios for the API. Ratios larger than 250 mL were assigned a classification as "not highly soluble," values <250 mL were assigned a classification as "highly soluble."

[a] See Table 4 for buffers.

medium (e.g., organic solvent or mobile phase) to prevent precipitation at room temperature. An appropriate dilution factor was determined in preliminary tests to guarantee that the measured concentration would fall within the validated linear calibration range. For all APIs, a dilution factor between 2 and 100 proved adequate. The pH of the remaining filtrate was checked, and any changes compared with the initial pH of the buffer were recorded.

The amount of dissolved drug in each sample was quantified via high-pressure liquid chromatography (HPLC) analysis with ultraviolet (UV) spectrometric detection. The injection volume was 20 µL, and 2 replicates were performed for each sample. The HPLC systems used consisted of a Hitachi LaChrom pump (LaChrom Elite L-2130 or LaChrom L-7110, respectively), an autosampler (L-2200/L-7200) an UV-detector (L-2400/7400) and a data integrator/organizer unit (D-7000). One system also contained a column oven (VDS optilab). LiChroCART® cartridges filled with LiChrospher® 100 RP-18, LiChrospher® 100 RP-18e, or LiChrospher® 100 RP-8e with 5 µm particle size (Merck Milipore, Darmstadt, Germany) of 2 different lengths (125 mm or 250 mm) were used for analysis. Further details on the HPLC methods such as composition of mobile phase, flow rate, column temperature, run time, retention time, and detection wavelength can be found in Table 5. Each method was validated for the respective API in accordance with the International Conference on Harmonisation guideline Q2(R1),[23] focusing on linearity, repeatability, limit of detection, and limit of quantification.

Permeability Data

To obtain permeability data for BCS classification, a literature search was performed in the bibliographic database PubMed (www.ncbi.nlm.nih.gov, accessed October 20, 2017). The international nonproprietary name of the respective API was searched in combination with one or more of the following key words: absorption, BCS, bioavailability (BA), fraction absorbed, mass balance, perfusion, permeability, pharmacokinetics, and radiolabeled. Permeability data were also obtained from the medical products professional information and the commentary on the European pharmacopoeia for the respective APIs, as well as from the primary sources of permeability data cited in these documents.

Classification of the APIs as "highly permeable" or "not highly permeable" was based on literature permeability or BA data indicative of fraction absorbed *in vivo* ≥85%, in accordance with the guidance documents published by FDA, EMA, and WHO.[6-8]

Results

The results of the solubility studies are shown in Table 6. When the amount of API weighed into the Uniprep™ syringeless filters completely dissolved in 3 mL of buffer solution, the resulting concentration (which represents the minimum solubility of the API) is listed. In all other cases, the mean solubility value and standard deviation calculated from the concentration of API in the saturated solutions at equilibrium sampled at each pH is stated. The D/S ratio was calculated under consideration of the highest dose strength of the pure API (free base or acid, respectively) listed on the 20th version of WHO EML[4] (see first column of Table 6). An API was considered "highly soluble" when the D/S ratio was ≤250 mL at all pH values examined, in accordance with the BCS criteria established by Amidon et al.[5]

Figure 2 shows the D/S ratios of the APIs classified as "highly soluble." With the exception of dexamethasone and cyclizine, the

Table 4
Buffer Compositions Used in Media for Solubility Studies

Buffer	Application
Hydrochloric acid buffer pH 1.2 (5.17.1 Ph.Eur. 8.0)	Amiodarone hydrochloride, atazanavir sulfate, dexamethasone, enalapril maleate, folic acid, hydroxychloroquine, medroxyprogesterone acetate, mifepristone, oseltamivir phosphate, ribavirin, rifabutin
Hydrochloric acid pH 1.2	Cyclizine, emtricitabine, mesna, morphine sulfate pentahydrate, succimer
Phosphate buffer pH 3.0 *R1*[a] (Ph.Eur. 8.0)	Folic acid
Acetate buffer pH 4.5 *R* (Ph.Eur. 8.0)	All substances
Phosphate buffer pH 6.8 *R1* (Ph.Eur. 8.0)	All substances

[a] Buffer with pH close to the solubility minimum of folic acid.

Table 5
HPLC Analysis of the APIs Studied

API	Column and Dimensions	Mobile Phase (V/V)	Flow Rate (mL/min)	Temperature (°C)	Detection Wavelength (nm)	Run Time/Retention Time (min)
Amiodarone hydrochloride	RP-18 (5μm) 125 × 4 mm	Phosphate buffer pH 3.0 R1 (Ph. Eur.)/acetonitrile (1:4)	2.0	40	240	7.0/3.2
Atazanavir sulfate[a]	RP-18e (5μm) 250 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 2.5 (1:1)	1.5	25	288	6.0/3.5
Cyclizine hydrochloride[b]	RP-18 (5μm) 125 × 4 mm	Acetonitrile/potassium dihydrogen phosphate 0.05 M pH 4 (1:1)	1.5	50	225	5.0/1.8
Dexamethasone	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	241	5.0/1.3
Emtricitabine	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	0.75	30	280	5.0/1.5
Enalapril maleate	RP-18 (5μm) 125 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	0.75	25	255	6.0/3.2
Folic acid[c]	RP-8e (5μm) 250 × 4 mm	Methanol/Phosphate buffer pH 6.3 (12:88)	0.6	25	280	30.0/8.2
Hydroxychloroquine sulfate	RP-18 (5μm) 125 × 4	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	1.0	25	255	17.0/2.5
Medroxyprogesterone acetate	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	241	5.0/2.6
Mesna[d]	RP-18 (5μm) 125 × 4 mm	Acetonitrile/phosphate buffer pH 2,3 (2:3)	1.0	25	235	4.0/1.0
Mifepristone	RP-18e (5μm) 250 × 4 mm	Acetonitrile/phosphate buffer pH 2.5 (1:1)	1.0	25	260	8.0/4.2
Morphine sulfate pentahydrate	RP-18 (5μm) 125 × 4 mm	Acetate buffer pH 4/acetonitrile (2:3)	0.75	30	280	10.0/1.85
Oseltamivir phosphate	RP-18 (5μm) 125 × 4 mm	Acetonitrile/ammonium phosphate buffer pH 3.5 0.2% (1:2)	1.0	25	230	6.0/1.8
Ribavirin[e]	RP-18e (5μm) 250 × 4 mm	Phosphate buffer pH 4.7	1.0	25	207	10.0/4.4
Rifabutin[f]	RP-18e (5μm) 125 × 4 mm	Acetonitrile/ammonium acetate solution pH 4.0 (1:1)	1.0	25	275	15.0/6.2
Succimer = meso-2, 3-dimercaptosuccinic acid	RP-18 (5μm) 125 × 4 mm	Deionized water/acetonitrile (1:4)	1.0	25	255	5.0/1.0

[a] Method adopted from Berlin et al.[17]
[b] The mobile phase was similarly composed as described by El-Gindy et al.[18] Flow rate was obtained from the same publication.
[c] Method adopted from Ph. Eur. 8.0.[19]
[d] Method adopted from Ph. Eur. 8.0.[20] Composition of the mobile phase was modified and a different column length was used.
[e] Method adopted from Belal et al.[21]
[f] Method adopted from Sangshetti et al.[22]

D/S ratios of all APIs classified as "highly soluble" depicted in Figure 2 were based on the observed minimum solubility. Figure 3 shows the D/S ratios of the APIs classified as "not highly soluble."

Table 7 presents the resulting BCS classification of all APIs included in this study, based on permeability data obtained from the literature and on the measured solubility of the highest dose strength listed on the current 20th version of the EML.[4]

Discussion

Solubility Classification and Eligibility for Biowaiver Procedure

Nine of the APIs examined in the present study were conclusively classified as "highly soluble," namely cyclizine, dexamethasone, emtricitabine, enalapril maleate, hydroxychloroquine sulfate, mesna, morphine sulfate pentahydrate, oseltamivir phosphate, and ribavirin. They demonstrated solubility values that would not lead to a change in the solubility classification of the particular drug even if the highest single therapeutic dose would be used for calculation instead of the highest dosage form strength listed on the EML. Considering that the solubility that was determined for most of the highly soluble compounds is a minimum value, the true D/S ratios are expected to be even lower than the ones shown in Figure 3 and Table 6. Furthermore, none of the compounds classified as "highly soluble" showed stability problems in the compendial buffers used. These 9 APIs are therefore possible candidates for a BCS-biowaiver procedure according to the WHO guidance document,[8] as they are either BCS I or BCS III compounds, depending on their permeability classification.

We note that in addition to the BCS I/III classification, further requirements have to be met for IR solid oral dosage forms containing highly soluble APIs to be eligible for a BCS-biowaiver procedure as stated the WHO guidance.[8] Depending on the BCS class, certain considerations regarding excipients and interpretation of the dissolution results have to be followed. Drug products containing BCS I APIs should use well-established excipients in usual amounts with no known influence on the absorption process. In comparative dissolution testing with an appropriate reference product, both the reference product and the multisource product to be approved have to release ≥85% of the total drug amount in 15 min (very rapidly dissolving) or in 30 min (rapidly dissolving), in which case there must be an additional comparison of the dissolution profiles via the f_2-test. Dissolution is carried out preferably with the United States Pharmacopoeia II apparatus operating at 50 rpm in ≤900 mL dissolution media of pH 1.2, 4.5, and 6.8. For drug products containing BCS III APIs, all excipients used should be qualitatively the same and quantitatively very similar to the reference product, and both drug products have to show very rapid dissolution under the conditions stated previously. In addition, a risk-benefit evaluation is conducted, taking into account the therapeutic index of the drug as well as the possible risk for public health if approval of a product which is actually bioinequivalent is erroneously granted via a BCS-biowaiver procedure. A complete overview of all the points addressed in an assessment of the feasibility of a biowaiver approval

Table 6
Solubility Values and Classification of the APIs According to BCS-Biowaiver Solubility Criteria

Drug (Dose)[a]	pH	Solubility (mg/mL)[b] (Mean ± SD)	Dose/Solubility-Ratio (mL)[b] (Mean ± SD)	Solubility Classification[c]
Amiodarone hydrochloride (400 mg)	1.2	$3.4 \pm 0.3 \times 10^{3}$	$125 \pm 11 \times 10^{3}$	Not highly soluble
	4.5	>5.0	<85	
	6.8	$1.02 \pm 0.03 \times 10^{3}$	$415 \pm 12 \times 10^{3}$	
Atazanavir sulfate (300 mg)	1.2	2.51 ± 0.11	136 ± 11	Not highly soluble
	4.5	$5.21 \pm 0.03 \times 10^{3}$	$65.5 \pm 0.4 \times 10^{3}$	
	6.8[d]	$<1.0 \times 10^{3}$	$>0.34 \times 10^{6}$	
Cyclizine (50 mg)	1.2	>3.75	<13.2	Highly soluble
	4.5	>3.94	<12.8	
	6.8	1.731 ± 0.019	28.9 ± 0.3	
Dexamethasone (4 mg)	1.2	$99.3 \pm 2.7 \times 10^{3}$	40.3 ± 1.1	Highly soluble
	4.5	$220 \pm 4 \times 10^{3}$	18.2 ± 0.3	
	6.8	$66.8 \pm 1.8 \times 10^{3}$	59.9 ± 1.7	
Emtricitabine (200 mg)	1.2	>3.35	<60.6	Highly soluble
	4.5	>3.87	<51.3	
	6.8	>3.86	<51.3	
Enalapril maleate (5 mg)	1.2	>5.13	<1.27	Highly soluble
	4.5	>5.23	<1.25	
	6.8	>5.13	<1.27	
Folic acid (5 mg)	1.2[e]	$15.95 \pm 0.22 \times 10^{3}$	314 ± 5	Not highly soluble
	3.0	$1.46 \pm 0.04 \times 10^{3}$	$3.42 \pm 0.09 \times 10^{3}$	
	4.5	$63.6 \pm 0.7 \times 10^{3}$	78.6 ± 0.8	
	6.8	>6.47	<0.773	
Hydroxychloroquine sulfate (200 mg)	1.2	>4.83	<53.5	Highly soluble
	4.5	>4.97	<52.1	
	6.8	>4.97	<52.1	
Medroxyprogesterone acetate (5 mg)	1.2	$0.9 \pm 0.4 \times 10^{3}$	$7 \pm 4 \times 10^{3}$	Not highly soluble
	4.5	$6.1 \pm 0.4 \times 10^{3}$	$0.82 \pm 0.05 \times 10^{3}$	
	6.8[d]	$<0.1 \times 10^{3}$	$>0.05 \times 10^{6}$	
Mesna (600 mg)	1.2	>3.56	<166.7	Highly soluble
	4.5	>4.11	<146.3	
	6.8	>3.52	<171.4	
Mifepristone (200 mg)	1.2	>4.57	<43.8	Not highly soluble
	4.5	$69.0 \pm 2.6 \times 10^{3}$	$2.90 \pm 0.11 \times 10^{3}$	
	6.8[d]	$<1 \times 10^{3}$	$>0.2 \times 10^{6}$	
Morphine sulfate pentahydrate (10 mg)	1.2	>4.39	<3.03	Highly soluble
	4.5	>3.98	<3.34	
	6.8	>3.79	<3.51	
Oseltamivir phosphate (75 mg)	1.2	>4.90	<20.2	Highly soluble
	4.5	>4.87	<20.3	
	6.8	>4.97	<20.2	
Ribavirin (600 mg)	1.2	>4.73	<127	Highly soluble
	4.5	>4.87	<123	
	6.8	>4.93	<122	
Rifabutin (150 mg)	1.2[e]	0.48 ± 0.07	$0.31 \pm 0.04 \times 10^{3}$	Not highly soluble
	4.5	3.13 ± 0.05	47.9 ± 0.7	
	6.8	$54 \pm 6 \times 10^{3}$	$2.82 \pm 0.27 \times 10^{3}$	
Succimer (100 mg)	1.2[e]	1.06 ± 0.16	96 ± 14	Not highly soluble
	4.5[e]	0.29 ± 0.03	$0.35 \pm 0.04 \times 10^{3}$	
	6.8[e]	1.3 ± 0.5	85 ± 28	

SD, standard deviation.

[a] The listed dose strengths are the highest strengths found on the 20th WHO EML[4] and refer to the free base or acid, respectively.

[b] If the excess amount weighed into the samples was completely dissolved at the end of the 24-h solubility study, a minimum solubility and maximum dose/solubility-ratio calculated from the sample with the least amount of drug weighed into the Uniprep™ syringeless filters is presented and indicated by ">" before the solubility value or "<" before the dose/solubility-ratio, respectively.

[c] Classification is based on the dose strengths in the first column (corrected for the respective salt form) divided by the experimentally obtained solubility values. A dose/solubility-ratio <250 mL corresponds to a classification as "highly soluble."

[d] Atazanavir sulfate, medroxyprogesterone acetate, and mifepristone showed solubility values below the limit of quantification at pH 6.8. The solubility value presented equals the limit of quantification for the respective API and minimum dose/solubility-ratios are presented.

[e] Folic acid and rifabutin showed degradation at pH 1.2, succimer showed noticeable degradation at all pH values.

can be found in the various published biowaiver monographs that are available from the web site of the International Pharmaceutical Federation at: *http://www.fip.org/bcs_monographs*.

The remaining 7 APIs investigated, namely amiodarone hydrochloride, atazanavir sulfate, folic acid, medroxyprogesterone acetate, mifepristone, rifabutin, and succimer, were classified as "not highly soluble." All of them, except for succimer (see Degradation Challenges section), failed to comply with the solubility criteria by at least a 10-fold difference. It is worth noting that 5 of these 7 APIs had their lowest solubility values at pH 6.8. Since this pH value represents the physiological environment of the small intestine, poor solubility at pH 6.8 could potentially lead to BA problems *in vivo* due to slow dissolution behavior or precipitation after an initial dissolution in the stomach and therefore reduced availability of dissolved drug substance for absorption. Independent of their permeability classification, an approval of drug products containing these APIs via the BCS-biowaiver is not currently possible according to any of the various regulatory guidances.

Most of the APIs demonstrated pH versus solubility profiles in line with expectations based on the presence or absence of acidic and basic functional groups (e.g., mifepristone, a basic molecule with a pKa of 4.89 shows an increase in solubility when pH

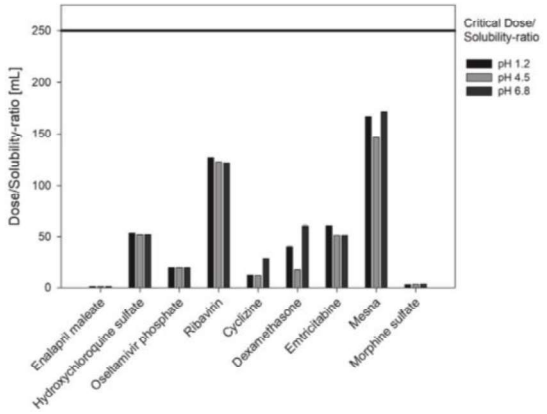

Figure 2. Dose/solubility-ratios of APIs classified as "highly soluble."

decreases). However, amiodarone hydrochloride, dexamethasone, and medroxyprogesterone acetate deviated from the expected behavior. Based on the molecular structure of amiodarone, an increase of solubility with decreasing pH is to be expected due to the basic tertiary amine side chain. As observed in our experiments, amiodarone demonstrated high solubility at pH 4.5 and poorer solubility at pH 1.2 and 6.8. The surprisingly poor solubility at pH 1.2 might be explained by common ion effect, as the salt form of amiodarone used in the experiments is a hydrochloride, and the media also contains chloride ions, thus reducing the degree of dissociation of the salt and the solubility. Dexamethasone and medroxyprogesterone acetate are neutral molecules; and therefore, no influence of media pH on solubility is to be expected. However, both APIs demonstrate the highest solubility at pH 4.5 and lowest solubility at pH 6.8. The observed solubility values, while differing from each other, reside in the same order of magnitude. A visual interaction with the buffer components, for example, the formation of precipitates, was not observed nor was there any change in the appearance or number of peaks in the chromatogram. In any case, measurements at all 3 pH values conclusively indicate high solubility for dexamethasone and poor solubility for medroxyprogesterone acetate, respectively.

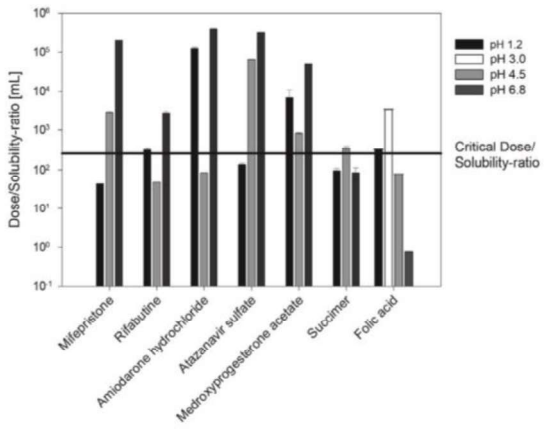

Figure 3. Dose/solubility-ratios of APIs classified as "not highly soluble."

BCS Classification and Possible Influence of Dose Strength Changes

The BCS classifications depicted in Table 7 were obtained using the experimentally determined solubility values and permeability data from the literature. To facilitate comparison with other, previously established classifications of the APIs, we added classifications available from the literature in column 5 of Table 7. The method adopted for classifying varied among the cited publications. For solubility classification, some authors used, whenever possible, solubility data found in the literature obtained from experiments using physiologically relevant conditions (pH 1.2-6.8, 37°C),[9,31,45] whereas others used aqueous solubility data at room temperature without specification of pH,[28-30,39,40] for example, obtained from the United States Pharmacopoeia solubility definitions or the Merck Index. One publication even relied on calculated solubility data derived from physicochemical properties.[29] Regarding the permeability classification, some authors used fraction absorbed and BA data found in the literature,[9,45] others relied solely on *in silico* data correlated to fraction absorbed values,[28,29,39,40] one group used CaCo-2 apparent permeability data for classification,[30] and one group used the Biopharmaceutics Drug Disposition Classification System[76] with ≥70% extent of metabolism as the criterion for high permeability.[31]

Although different approaches for establishing a BCS classification were used, the resulting BCS classifications are mostly in accordance with each other (Table 7), especially with respect to the solubility classifications. The only exceptions were medroxyprogesterone acetate, mifepristone, and folic acid, which were classified as "highly soluble" or "not highly soluble," depending on the reference cited.

Medroxyprogesterone acetate is listed as "highly soluble" in the document "Proposal to waive *in vivo* bioequivalence requirements for WHO Model List of Essential Medicines immediate-release, solid oral dosage forms (Annex 8)"[45] based on its solubility in water at room temperature. Because of the low dose of 5 mg, using the solubility definition "practically insoluble in water (<0.1 mg/mL)" from Clarke's analysis of drugs and poisons still leads to a classification as highly soluble with a D/S ratio of 50 mL. This D/S ratio is an underestimation, as the solubility at pH 6.8 is much lower than 0.1 mg/mL (Table 6), and medroxyprogesterone acetate is therefore correctly classified as "not highly soluble."

Mifepristone was classified provisionally as BCS III or IV by the WHO[45] because no solubility data were available at that time to establish a reliable solubility classification. According to our experiments, mifepristone is clearly to be classified as "not highly soluble" and therefore deemed a BCS IV compound (Table 7).

Folic acid was classified as "not highly soluble" by several authors.[9,31,40] In contrast, others have deemed folic acid to be a highly soluble compound.[28,30,39,45] The pH range considered for classifying the solubility can explain this divergence. If only solubility data in pure water or at pH 1.2, 4.5, or 6.8 are considered, folic acid will be incorrectly classified as borderline highly soluble. But when the solubility at pH 3.0 is taken into consideration, folic acid is clearly classified as not highly soluble because at this pH, the D/S ratio is ≥3 L for a dose of 5 mg (Table 6). This example demonstrates the importance of not only relying on solubility data in pure water or the "standard" pH values proposed in the guidance documents but also solubility values at the pH where the solubility is expected to be lowest.

In the various guidance documents, different definitions of the dose strength to be used for establishing the D/S ratio can be found.[6-8] Although the FDA recommends the highest dosage strength of a marketed IR drug product to be used,[6] the WHO and EMA guidance define that the D/S ratio should be established with

Table 7
BCS Classification of the APIs Based on Measured Solubility Data and Permeability Data From the Literature

Drug Name (WHO EML Dose)	Solubility	Permeability	BCS Class[a]	Previous Classification(s)	Comments
Amiodarone hydrochloride (400 mg)	Low	Low[24-27]	**IV**	II[28-31]	Incomplete absorption (~20%-50%), P-gp inhibitor
Atazanavir sulfate (300 mg)	Low	Low/high[17,32,33]	**IV**/II	IV,[30] II/IV,[17] II[31,33]	Nonlinear pharmacokinetics (range 100-1200 mg), inconclusive f_a data, P-gp Efflux
Cyclizine (50 mg)	High	High/low[34,35]	**I**/III	I[29-31]	No reliable permeability data
Dexamethasone (4 mg)	High	High[36-38]	**I**	III,[30] III/I,[9,39,40] I[28,29,31]	Incomplete BA due to presystemic elimination rather than poor absorption
Emtricitabine (200 mg)	High	High[41]	**I**	III[30,31]	Oral BA > 90%, linear kinetics (100-1200 mg)
Enalapril maleate (5 mg)	High	Low[42-44]	**III**	III,[30,40,42,45] III/I,[28] I[29,39]	~60%-70% of a dose is absorbed
Folic acid (5 mg)	Low	Low/high[46-51]	**IV**/II	IV,[40] IV/II,[9] II,[31] III,[28,30,39] III/I[45]	No reliable data for doses >5 mg, saturable active transport
Hydroxychloroquine sulfate (200 mg)	High	High/low[52,53]	**I**/III	I[30,31]	Rapid and almost complete absorption, BA ~ 67%-74%
Medroxyprogesterone acetate (5 mg)	Low	Low[54]	**IV**	IV,[31] IV/II,[30,39] II,[28,40] III/I[45]	Extent of oral absorption is <10%, positive food effect
Mesna (600 mg)	High	Low/high[55,56]	**III**/I	III/I,[30] I[31]	70% of an oral dose is found in urine (compared with intravenous data)
Mifepristone (200 mg)	Low	Low[57,58]	**IV**	IV/III[45]	Fraction absorbed ~70%, BA ~ 40%, nonlinear pharmacokinetics above 100 mg
Morphine sulfate (10 mg)	High	High/low[59-62]	**I**/III	III,[29,30] III/I,[9,28,40,45] I[31]	BA ~ 30%, high FPE, 90% of a dose is metabolized and found in urine
Oseltamivir phosphate (75 mg)	High	High[63-66]	**I**	III/I,[28,40] I[30,31]	High FPE (~70%-80% of a dose is metabolized in the liver), high BA of metabolized drug (>80%)
Ribavirin (600 mg)	High	Low/high[67-69]	**III**/I	III,[28,30,40] III/I,[69] I[31]	High intestinal FPE, active transport, positive food effect
Rifabutin (150 mg)	Low	Low[70-72]	**IV**	IV,[30] II[31]	Low BA, induces own metabolism, ~ 50%-60% metabolized in urine; highly variable; significant degradation in acidic media
Succimer (100 mg)	Low	Inconclusive[73-75]	**IV**/II	Not classified	Literature data were inconclusive.

FPE, first pass effect.
[a] BCS classifications depicted in **boldface** are the preferred classifications suggested by the authors of this article.

the highest single dose (which could consist of administering multiple dosage forms to achieve a required dose).[7,8] The D/S ratios in Table 6 were calculated based on the highest dose of an IR drug product listed on the WHO EML, which is usually the highest dosage strength of the drug product. A change in the dose definition can only have an impact on the highly soluble compounds, as the D/S ratio can only become larger and not lower. Even when applying the WHO/EMA definition of "dose," all the APIs classified as "highly soluble" by the FDA definition in the present study would remain in that category, further indicating that the solubility classification established for the APIs included in our study is reliable independent of the dose definition used.

The influence of the definition of "dose" on the BCS classification was also investigated in a review of published biowaiver monographs.[77] The impact of the difference between the 2 definitions varied among the 24 individual APIs; as for some, the dose considered did not change (highest single dose = highest dosage strength, 6 APIs), whereas for other APIs, the highest single dose was as much as 5 times the highest dosage strength (e.g., ethambutol hydrochloride, isoniazid).[77] Of the BCS classifications of 24 APIs examined, 2 changed when using the EMA/WHO rather than the FDA dose definition and 2 had to be reevaluated.[77]

Choice of Experimental Conditions and Challenges

The experimental conditions of a solubility study must be chosen carefully. One crucial aspect is the influence of the solid state form of the evaluated substance on the solubility. Different polymorphic forms might show different values for solubility. With respect to APIs with several polymorphic forms, it is recommended that the solid state form of the chosen material is identified in the solubility report. Since a full solid state characterization of the examined APIs was outside the scope of this study, pharmacopoeial reference standards were chosen as study material wherever possible (Table 2).

In contrast to the proposed method for solubility determination in the various guidance documents,[6-8] we determined a minimum solubility after 24 h rather than a thermodynamic equilibrium solubility for the highly soluble APIs. Since some APIs on the EML are rather expensive, a more cost-effective method was chosen to establish solubility classifications. The scaled-down approach, adopted from Glomme et al.,[11] yielded various advantages when compared with the conventional shake-flask method. In most cases, about 15 mg of API per sample was sufficient to establish a reliable solubility classification. Determining the equilibrium solubility of highly soluble compounds would have sometimes required using more than 600 mg per sample to exceed the maximum dose strength—even using the scaled-down experiment—for example, for ribavirin. Using such excessive amounts of API to determine the equilibrium solubility is not only wasteful but can also lead to a change in the pH of the buffer solutions if the drug has acidic or basic properties, as is the case for several of the APIs investigated in our study. The resultant high concentration of dissolved drug would likely exceed the buffer capacity of media and would therefore require adjustment of the pH during the incubation period, changing the total volume and introducing an additional source of variability.

In addition to using a scaled down version of the shake-flask method, we selected a 24-h time frame to determine the solubility. A 24-h time point was selected because experience in our laboratories has shown that most APIs achieve their equilibrium solubility within this time frame. Furthermore, since the physiological transit time of drugs through the absorptive compartments of the gastrointestinal tract is rarely more than 24 h, extending the solubility determinations to more than 24 h seems unnecessary. Figures 2 and 3 show that relevant and reliable values were obtained for all APIs using the (minimum) solubility at 24 h approach, with the exception of succimer and ribafutin. In the case of succimer and ribafutin, the solubility measurements were complicated by their degradation under the experimental conditions. In these cases, a considerably shorter time frame for the solubility measurement, for example corresponding to upper gastrointestinal transit time, may be more appropriate.

Degradation Challenges

During the 24-h incubation at 37°C, degradation was observed for 3 drugs, which were later categorized as "not highly soluble": folic acid, rifabutin, and succimer. In additional studies to quantify the extent of degradation, it was found that after 4 h at pH 1.2°C and 37°C, about 5% of the total amount of folic acid in a solution of known concentration and about 30% of the total amount of rifabutin in a solution of known concentration had degraded. The relative extent of degradation was estimated for each API from its peak area in the chromatogram at each individual time point divided by the peak area at the beginning of the degradation study (t = 0 h). Degradation rates of folic acid and rifabutin at acidic pH values observed in this study are in accordance with results of degradation studies found in the literature.[22,78-81] Succimer dissolved directly after immersion in the different buffers, but a large amount had sedimented after the 24-h incubation. Immediately after adding the media, hydrogen sulfide was detected organoleptically, especially with the more acidic media. In contrast to succimer solutions prepared in organic solvents, these samples showed degradation peaks in the HPLC analysis. It was inferred that succimer undergoes hydrolysis and that the obtained values, although indicating that succimer is not highly soluble, do not reflect the true thermodynamic solubility of succimer. The instability of succimer in aqueous media at physiological pH values, the fact that no reliable permeability data are available in the open literature and that it shows borderline solubility behavior make it impossible to reliably classify succimer according to the BCS. Following a worst-case approach, succimer is conservatively classified as BCS IV, and thus unsuitable for a BCS-biowaiver approval.

The possibility of degradation during the solubility determination requires a stability indicating analytical procedure such as analysis via HPLC, which is also recommended in the FDA draft guidance document.[6] Analytical methods solely based on UV-Vis spectroscopy may lead to biased results if the investigated drug demonstrates instability in the test media.

The various guidance documents state no specific consequence for the BCS classification of an API if degradation during solubility measurement occurs. In the solubility section of the FDA draft guidance document, it is stated that the occurrence of degradation should simply be reported,[6] as degradation may also have an influence on the amount of drug available for absorption. In the section discussing permeability of the same document, it is stated that instability in the gastrointestinal tract should be taken into consideration. Here, degradation to an extent ≥5% is considered significant,[6] and the FDA recommends degradation studies to be carried out in simulated gastric or intestinal fluids at 37°C for a period of 1 h or 3 h, respectively.[6] When a compound shows a large degree of degradation in acidic media for example rifabutin (≥30% in 4 h at pH 1.2), it is to be assumed that this could also influence the permeability criterion as defined by the BCS. In fact, if the compound shows degradation to an extent greater than 15% under conditions corresponding to those before or at the site of absorption, it is reasonable to infer that the fraction of dose absorbed *in vivo* cannot be equal or higher than 85%. For this reason, we propose that an otherwise highly permeable drug showing degradation to an extent ≥15% over 1 h in simulated gastric fluid (reflecting a rather slow gastric emptying time in the fasted state) at a temperature of 37°C (the conditions stated in the FDA draft guidance document[6]), the drug should be classified as "not highly permeable." For the investigation of degradation under intestinal conditions, the FDA recommendation of experiments in simulated intestinal fluid at 37°C for 3 h seems appropriate. Since degradation to an extent of ≥15% in 3 h measured *in vitro* could be compensated or even negated by rapid absorption *in vivo*, no reliable assumption can be made here about the fraction of the dose available for absorption. The potential influence of intestinal degradation of an API has therefore to be discussed individually for each API. Both degradation experiments should be carried out using stability indicating dissolution testing, a method which is described and implemented in the biowaiver monograph for acetylsalicylic acid.[82]

Impact of Degradation on the BCS Solubility Classification

Significant degradation during the 24-h solubility measurement in any of the media can have an influence on the resulting solubility classification. If decomposition occurs, the 24-h solubility approach could yield either higher or lower solubility values compared with solubility measurements of shorter duration and might therefore lead to the wrong BCS classification. For APIs that show degradation at pH values that are relevant for the BCS classification (i.e., pH 1 to pH 6.8), additional solubility measurements should be carried out. The appropriate time period for the additional solubility experiments can be inferred from the degradation studies discussed in the previous section: for substances showing degradation a pH 1.2, the maximum time period for the supplementary solubility determination should be 1 h, as a longer exposition to this media pH is unlikely *in vivo*. For degradation at other pH values, a maximum of 3 h as a time period for additional solubility experiments is reasonable because this time frame corresponds to approximately the period in which the majority of the uptake of an API in an IR formulation from the small intestine is expected.

If a drug shows a rate of degradation higher than 15% in 1 h under gastric conditions or 3 h under intestinal conditions, the duration of the solubility experiments should be no longer than the time required for 15% decomposition (in other words, until the time when 85% of the drug is still intact). For the APIs investigated in our study, such additional solubility experiments were not necessary even for the APIs showing degradation such as folic acid and rifabutin. Although both of these APIs degraded in acidic media (pH 1.2), they had already shown poor solubility at other pH values and thus it could be concluded that they were not "highly soluble" within the BCS definition.

Nevertheless, consideration of degradation might be important for other APIs, which are highly soluble over the entire pH range required for BCS. For example, the impact of hydrolysis on solubility measurement is discussed in the biowaiver monograph of acetylsalicylic acid.[82] In that publication, the authors chose a duration of 15-45 min (depending on the media pH) for the solubility experiments to ensure that the extent of degradation during the study would be less than 2%. If a 24-h solubility determination would have been used, the degradation of acetylsalicylic acid would

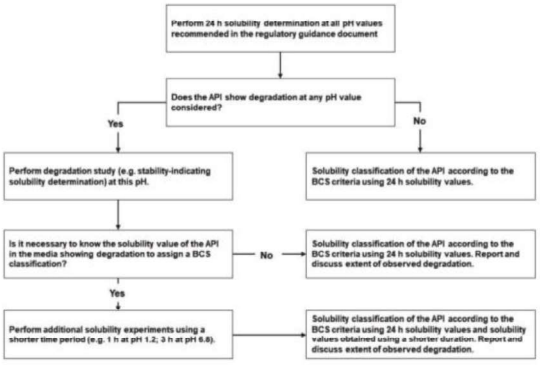

Figure 4. Decision tree for performing solubility determinations in the context of the BCS-based biowaiver.

have been almost complete, resulting in an underestimation of the solubility and potentially in an erroneous solubility classification as "not highly soluble." For acetylsalicylic acid, it would have also been possible to determine the solubility over 1 h at pH 1.2 and over 3 h at pH 6.8, as recommended earlier in this paragraph because, using a worst-case assumption, the time to 10% degradation is 3.17 h at pH 6.8 for acetylsalicylic acid, indicating that more than 90% of the API would remain intact for at least 3 h.

An overview of the experimental procedure proposed in this section is depicted in Figure 4 as a decision tree.

Conclusions

The experimental study protocol elaborated in these studies, which is based on a miniaturized shake-flask method, is a fast and cost-effective approach for establishing a reliable solubility classification of APIs listed on the WHO EML in the context of the BCS-based biowaiver and enabled all APIs studied to be clearly classified into 1 of the 2 solubility categories. Of the 16 APIs, 3 were assigned to BCS class I, 1 to class III and 4 to class IV. For 8 APIs, permeability could not be well defined from the literature, resulting in 5 class I/III classifications and 3 class II/IV classifications. The resulting solubility and BCS classification were in accordance with other, previously proposed, classifications, suggesting that although the current results were obtained using a scaled-down method and that experiments were conducted over 24 h rather than requiring thermodynamic equilibrium to be reached, the scaled-down methodology provides an accurate BCS classification. In particular, using the "minimum solubility" approach can dramatically cut down the amount of API required to obtain a solubility classification for "highly soluble" drugs while avoiding issues with maintenance of the target pH value when studying weak acids and bases. Thus, it is proposed that some flexibility in the determination of solubility for BCS purposes be allowed in future guidances. We would further like to emphasize the importance of stating the experimental conditions in conjunction with the solubility classification as either "highly soluble" or "not highly soluble," to enable calculations based on other "dose" definitions and to allow better assessment of the quality of the data.

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A.1.3.2 Publication 2

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Global Health Commentary

Biowaiver Monographs for Immediate-Release Solid Oral Dosage Forms: Folic Acid

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ABSTRACT

This work presents a review of literature and experimental data relevant to the possibility of waiving pharmacokinetic bioequivalence studies in human volunteers for approval of immediate-release solid oral pharmaceutical forms containing folic acid as the single active pharmaceutical ingredient. For dosage forms containing 5 mg folic acid, the highest dose strength on the World Health Organization Essential Medicines List, the dose/solubility ratio calculated from solubility studies was higher than 250 mL, corresponding to a classification as "not highly soluble." Small, physiological doses of folic acid (≤320 μg) seem to be absorbed completely via active transport, but permeability data for higher doses of 1-5 mg are inconclusive. Following a conservative approach, folic acid is classified as a Biopharmaceutics Classification System class IV compound until more reliable data become available. Commensurate with its solubility characteristics, the results of dissolution studies indicated that none of the folic acid products evaluated showed rapid dissolution in media at pH 1.2 or 4.5. Therefore, according to the current criteria of the Biopharmaceutics Classification System, the biowaiver approval procedure cannot be recommended for immediate-release solid oral dosage forms containing folic acid.

Introduction

Folic acid (vitamin B_9) metabolites are essential cofactors for human metabolic functions such as single carbon transfer reactions in the synthesis of nucleotides and metabolism of amino acids.[1] Therapeutically, folic acid is indicated for the treatment of vitamin B_9 deficiency, which can lead to megaloblastic anemia or hyperhomocysteinemia.[1,2] Furthermore, prophylactic supplementation of folic acid is widely recommended for women during the periconceptional period to prevent fetal neural tube malformations such as anencephaly and spina bifida.[3] The 19th World Health Organization (WHO) Model List of Essential Medicines lists folic acid as an antianemic drug in dose strengths ranging from 0.4 to 5 mg.[4]

In this monograph, the biopharmaceutical and clinical properties of folic acid as well as the risks associated with waiving pharmacokinetic bioequivalence (BE) studies to facilitate the approval of generic immediate-release solid oral dosage forms containing folic acid as the sole active pharmaceutical ingredient (API) are examined to determine whether a biowaiver approach is feasible and can be recommended for such formulations. To evaluate the risks associated with applying the biowaiver procedure to folic acid

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formulations, data available in the literature were reviewed and, where data were inconclusive or absent, supplementary experimental data were generated. Risks evaluated in this work include the probability of an incorrect decision of granting a biowaiver and its consequences from a public health point of view, as well as the risks for individual patients. This is consistent with the purpose and scope of the series of biowaiver monographs that have already been published for many other active pharmaceutical ingredients[5] and which are available online via www.fip.org/bcs.

The systematic approach to deciding whether to recommend a biowaiver procedure or not is referred to in annex 7 of the technical report series 992 released in 2015 and annex 8 of the technical report series 937 released in 2006 from the WHO[6,7] as well as in the guidances published by the American (United States Food and Drug Administration [FDA])[8] and European (European Medicines Agency)[9] regulation agencies. However, the biowaiver monographs are not aimed at merely applying these guidelines but rather critically evaluating the properties of the API and applying risk analyses to complement the Biopharmaceutics Classification System (BCS).

Methods

Literature Search

The method adopted was a review of the various guidances as well as scientific papers relevant to the biowaiver approach and folic acid properties. In the bibliographic databases PubMed (www.ncbi.nlm.nih.gov) and SciFinder (https://scifinder.cas.org), the keyword "folic acid" was searched in combination with one or more of the following keywords: absorption, bioavailability (BA), BE, carrier, dissolution, distribution, excretion, food, mass balance, metabolism, partition coefficient, permeability, pharmacokinetics, p*Ka*, polymorphism, protein binding, radiolabeled and solubility. Last date of access of the publicly available literature was March 2017.

Solubility Study

A study to investigate the pH-dependent solubility of folic acid was carried out at the Goethe University in Frankfurt am Main using a downscaled shake flask method previously described by Glomme et al.[10] Excess amounts (~20 mg per sample) of folic acid were weighed into UniPrep® syringeless filters and 3 mL of buffer solution was added. Buffer solutions used were the following Ph. Eur. buffers and media: hydrochloric acid medium pH 1.2,[11] phosphate buffer solution pH 3.0 *R*1, acetate buffer solution pH 4.5, and phosphate buffer solution pH 6.8 *R*1.[12]

The samples were then shaken at 45 rpm on an orbital shaker for 24 h at 37 ± 0.5°C and then filtered through the 0.45-μm PTFE filters integrated in the UniPrep system. All samples were prepared in triplicate under exclusion of light. The pH value of the buffer solutions was confirmed after addition to the API and at the end of the experiment and also adjusted to the nominal pH value after 4 h if needed. The samples were diluted with mobile phase and analyzed via HPLC with UV detection at 280 nm using the validated stability-indicating assay for folic acid described in the Ph. Eur. 8.0.[13] Although no specific pH was specified in the pharmacopoeial method, the mobile phase described in the method was adjusted to pH 7.2 to ensure that all folic acid in the sample would be in ionized form. The column used was a 250 mm LiChrospher® 100 RP-8e (5 μm) cartridge. The total amount of dissolved folic acid was calculated using the equation of a calibration curve ($R^2 = 0.999$) prepared with different dilutions of a stock solution of known concentration. The limit of quantification was 0.2 μg/mL.

General Characteristics

Three subunits can be identified in the molecular structure of folic acid (pteroyl-L-glutamic acid), represented in Figure 1: 6-methylpterin; 4-aminobenzoic acid, and glutamic acid.[14]

The generic term "folate" refers to a class of compounds which have similar chemical structures and nutritional activity compared to folic acid. The various naturally occurring folates exist primarily in the reduced form, differing in the substituent at position 5 or 10, respectively, as well as in the number of glutamic acid moieties bound to the pteroyl group. Five different possible substitutes (methyl, formyl, formimino, methylene, and methenyl) are known. Most naturally occurring folates show a side chain of 5 to 7 residues of glutamic acid, connected by peptide γ bonds.[15]

Therapeutic Indications

Orally administered folic acid in doses of 1-15 mg is indicated for the treatment of megaloblastic anemia that does not involve neurologic disorders. This form of anemia can be caused by a deficiency of either vitamin B_{12} or folic acid, so one must keep in mind the potential danger of inappropriately treating a vitamin B_{12}-deficient patient with folic acid. Therapy with folates neither prevents nor alleviates the neurologic defects that can be caused by vitamin B_{12} deficiency, which may progress further and can become irreversible.[1] Daily doses of 0.5-5 mg folic acid are indicated for the treatment of hyperhomocysteinemia when vitamin B_{12} deficiency is excluded as the cause.[2] At lower doses of 400–800 μg, folic acid is widely recommended as a supplement for women in the periconceptional period to prevent fetal neural tube defects such as anencephaly and spina bifida.[16] The beneficial effect of this prophylactic intake of folic acid is well established.[3]

It has also been suggested that folic acid may be effective in reducing the risk of certain heart and also psychiatric diseases, such as dementia.[17] Moreover, there are associations between plasma folate levels and prevention of certain types of cancer: it has been proposed that high levels of folate and vitamin B_6 in the plasma may reduce the risk of developing breast cancer,[18] and other studies have suggested that a decrease in folate plasma levels is associated with an increase in the risk of colon cancer.[19] By contrast, other studies have associated folic acid intake with the promotion of cancer development and progression.[20-22] Because evidence for an effect (either positive or negative) of folic acid on these diseases is not sufficiently supported by randomized controlled trials, a quantitative risk/benefit assessment for recommending a general prophylactic supplementation of folic acid is currently not possible.[3]

Therapeutic Index and Toxicity

In the European Food Safety Authority Meeting Report on folic acid, it is stated that the long-term supplemental intake of folic acid should not exceed 1 mg/d.[3] A recent review on the safety of folic acid also concludes that supplemental daily intake of up to 1 mg synthetic folic acid can be regarded as safe, as there is no evidence for an increased risk or undesired effects on diseases such as colorectal cancer linked to folic acid intake.[23] Even at high doses of up to 15 mg folic acid per day, there has not been any substantiated report of side effects.[24] The LD_{50} in mice was found to be 115 mg/kg and 10 g/kg after intravenous and oral administration, respectively, also suggesting low acute toxicity of folic acid.[1,25]

Taking into account the low acute toxicity of folic acid in doses up to 5 or 15 mg used in acute treatment of hyperhomocysteinemia or megaloblastic anemia, and the comparatively low doses of 400-800 μg used for prophylactic supplementation to compensate for

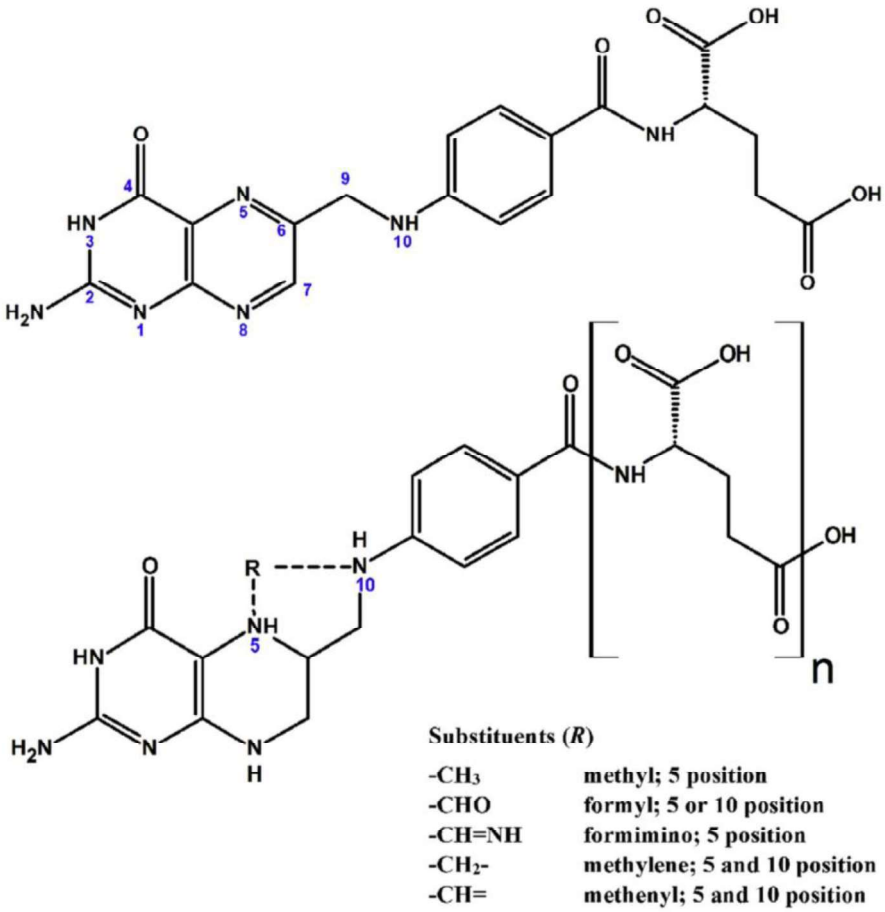

Figure 1. Upper structure: folic acid (pteroyl-L-glutamic acid); lower structure: natural folates occurring in food, modified from Forssén et al.[15]

vitamin deficiencies (e.g., during pregnancy), which are below the designated upper limit of 1 mg for daily supplemental intake, it can be concluded that folic acid is not a narrow therapeutic index drug.

Physicochemical Properties

Polymorphism

Santos et al.[26] reported that there are no polymorph forms for folic acid. However, Braga et al.[27] have subsequently shown that 2 different conformational, monotropically related polymorphs of folic acid dihydrate exist which differ only in the conformation of the glutamic acid moiety. However, Braga et al.[27] did not provide any information indicating differences in solubility values or dissolution behavior.

At room temperature, the drug has a crystalline form and a water content of 5%-8.5%. Complete degradation with no discernable transition between polymorph forms occurs at temperatures higher than 200°C: the adsorbed water is lost first, followed by decomposition of the glutamic acid moiety and the other constituents, leaving behind only an amorphous residue of carbon.[28,29]

pKa

Szakács and Noszái reported the following values for apparent dissociation constants, determined by pressure-assisted capillary electrophoresis at 25°C and at an ionic strength of 0.05 M: $pKa_1 = 2.38 \pm 0.04$, $pKa_2 = 3.46 \pm 0.03$, $pKa_3 = 4.98 \pm 0.03$, and $pKa_4 = 8.08 \pm 0.03$. They also compared the constants measured by pressure-assisted capillary electrophoresis to values obtained by other techniques. Those experiments were carried out at 25°C with an ionic strength of 0.15 M.[30]

A study of Sköld et al.,[31] using a combination of potentiometric and spectrophotometric (UV) techniques to determine p*Ka* values, reported the following values for folic acid: 2.16, 3.79, 4.47, and 7.90. Similar p*Ka* values are stated by Wu et al.,[32] with which they established a pH and temperature-dependent solubility model for folic acid.

Table 1 lists all previously measured p*Ka* values relevant to the physiological pH range reported in the respective publications. At pH values lower than pKa_1, folic acid is predominantly positively charged (protonated at N[1]) in aqueous solution and negatively charged at pH values above pKa_2 (deprotonated α-carboxylic acid group), resulting in the folic acid molecule having a minimum net charge at pH values between pKa_1 and pKa_2.

Solubility

Folic acid is listed in the Ph. Eur. 8.0 as practically insoluble in water.[13] Its aqueous solubility has variously been reported as 1.6 μg/mL at 25°C[33] or 0.1 mg/mL.[34] Sköld et al. reported a kinetic

Table 1
p*Ka* Values of Folic Acid Obtained From Literature Data

Reference	Method	c (mM)	pKa_1	pKa_2	pKa_3	pKa_4
Szakács and Noszál[30]	PACE[a]	≤0.01	2.38 ± 0.04	3.38 ± 0.03	4.83 ± 0.03	7.85 ± 0.03
	UV-Vis	0.03	(≈2.6)	–	–	7.98 ± 0.01
	^{1}H-NMR	1	–	–	–	8.01 ± 0.01
	Tit. pot. aq.[b]	1	–	–	–	7.96 ± 0.05
	Tit. pot. extr.[c]	2	–	3.34 ± 0.04	4.7 ± 0.2	7.9 ± 0.1
Sköld et al.[31]	Pot.-UV[d]	–	2.16	3.79	4.47	7.90
Wu et al.[32]	Various techniques	–	2.35 ± 0.1	3.46 ± 0.02	4.56 ± 0.03	8.38 ± 0.03

PACE, pressure-assisted capillary electrophoresis.
[a] Values obtained with an ionic strength of 0.05 M were converted to an ionic strength of 0.15 M to allow straight comparison with other experimental data.
[b] Potentiometric titration in aqueous solution.
[c] Potentiometric titration in DMSO-water, values extrapolated for aqueous solution.
[d] Combination of potentiometric and spectrophotometric techniques.

solubility of 30.0 μg/mL and an intrinsic solubility of 2.3 μg/mL.[31] Table 2 shows 3 sets of results obtained in buffer solutions at different pH values at 37°C. The first set of experiments was conducted in a pH range from 1 to 10 using 5 different buffers.[35] The second set of experiments was realized by Bellavinha et al.[36] and was obtained using a shake flask method and buffer solutions with pH values in accordance with the biowaiver guidance. The results were used to classify the solubility of folic acid in terms of the BCS. The study found the dose/solubility ratio for a dose of 5 mg of folic acid to be above 250 mL, which corresponds to a classification as "not highly soluble." Because the solubility data reported in the literature are inconsistent, a third set of results was obtained experimentally (see Methods section). The low solubility at pH 3.0 is in agreement with the results of Wu et al.,[32] who investigated the solubility of folic acid in water at different pH values and temperatures and found the lowest solubility to be at pH values close to 3 at 25°C, 30°C, and 40°C.

Figure 2 depicts a comparison of the solubility data for folic acid obtained by the various working groups. As described in the previous section, folic acid exists as a predominantly uncharged molecule in aqueous solution at pH values between 2.4 and 3.5, explaining the minimum of the pH-dependent solubility curve found by Wu et al.

Taking into account the low solubility at pH 1.2-4.5 with dose-solubility ratios above 250 mL, folic acid is classified as "not highly soluble."

Pharmaceutical Dosage Forms

The highest dose strength stated in the current WHO Essential Medicines List for folic acid tablets is 5 mg.[4] Approved medicinal products are available in dose strengths up to 1 mg in the United States[37] and up to 5 mg in Germany[38] and the UK.[39] Although there are formulations containing folic acid in combination with other active pharmaceutical ingredients commercially available, this monograph is concerned with immediate-release solid oral dosage forms containing only folic acid.

Table 2
Solubility of Folic Acid at 37°C Obtained From Experimental and Literature Data and the Correspondent Dose/Solubility Ratio (D/S) for a Dose Strength of 5 mg

Reference	Buffer Solution pH	Solubility (mg/mL)	D/S[a] (mL) 5 mg[b]
Younis[35]	1	29×10^{-3}	172.41
	3	0.840	5.95
	4	1.050	4.76
	7	5.330	0.93
	10	19.47	0.26
Bellavinha et al.[36]	1.2	9×10^{-3}	569.9
	4.5	19×10^{-3}	266.7
	6.8	4.340	1.15
Experimental results	1.2	15.95×10^{-3}	314.4
	3.0	1.46×10^{-3}	3.42×10^{3}
	4.5	63.6×10^{-3}	78.6
	6.8	≥6.47[c]	≤0.773[c]

[a] Critical limit: 250 mL.
[b] Highest dose according to the WHO Essential Medicines List.
[c] The amount of API was completely dissolved in all 3 samples, the value stated corresponds to the sample with the least amount of API weighed in.

Pharmacokinetic Properties

Absorption and Permeability

Folic acid is absorbed in the proximal segment of the small intestine and to a lesser extent in the lower intestines, with the absorption rate decreasing from jejunum to colon.[40,41] The uptake mechanism consists of 2 pathways: a pH-dependent, saturable carrier-mediated pathway at lower concentrations and a passive uptake via diffusion that is less efficient compared to active transport, due to folic acid being negatively charged and hydrophilic at physiological pH values.[42,43] The passive uptake mechanism is therefore only relevant at high intestinal folate concentrations.[44]

The folate carrier system in the intestines is mainly composed of reduced folate carriers (RFCs) and proton-coupled folate transporters (PCFTs). Considerable amounts of PCFT are located in the brush-border membrane of the proximal jejunum and duodenum.[42] PCFT is a high-affinity folate carrier with maximum transport activity at low pH values (~pH 5.5). The transport activity decreases as the pH increases and is almost negligible at pH 7.4. In contrast, RFCs show almost no activity below pH 6.5 and have a lower affinity for folic acid compared to PCFT.[42] RFCs are located at

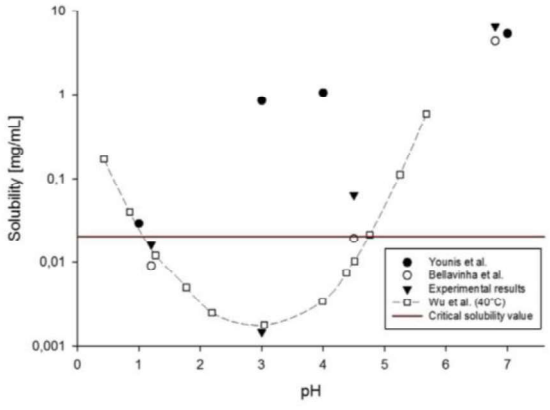

Figure 2. pH-dependent solubility of folic acid as determined in different studies.

the proximal section of the small intestine but are also present in the colonic mucosa and seem to mediate the uptake of reduced folates synthesized by colonic bacteria alongside passive diffusion and PCFTs.[40,45]

An ileostomy trial indicated that only about 10% of a labeled 200 μg dose of folic acid would reach the colon, with the rest of the dose being absorbed in the small intestine,[46] confirming the proximal jejunum and duodenum as the main site of absorption for orally administered folic acid. Nevertheless, the absorptive capacity of the colon is still sufficient for the uptake of a 400 μg supplemental dose of folic acid, as shown in patients with Roux-en-Y gastric bypass, an operation which precludes the uptake of folic acid in the duodenum and proximal jejunum.[40]

A study using 2 mg folic acid labeled with tritiated folic acid showed absorption values ranging from 65% to 95% (mean: 80%) in pregnant and nonpregnant subjects.[47] In a study with 17 healthy subjects conducted by Menke et al.,[48] the absolute BA of 5 mg orally administered folic acid was determined to be 76.2 ± 13.8%. The absolute BA was calculated using AUC values corrected for the individual predose serum level of folic acid after a 9-day saturation period with 5 mg orally administered folic acid per day and a successive 4-day washout period, which prevented nonlinear pharmacokinetic effects due to distribution and storage kinetics being affected by total body folate status.

A permeability study in CaCo-2 cells determined an apparent permeability (P_{app}) for folic acid of 1.7×10^{-6} cm/s, indicating moderate permeability when compared to mannitol ($P_{app} = 0.5 \times 10^{-6}$ cm/s) and caffeine ($P_{app} = 34 \times 10^{-6}$), the reference compounds for low and high permeability, respectively.[49]

The fraction absorbed of small physiological doses of folic acid (≤320 μg) as determined in mass balance studies is stated to be ~90%,[50-53] concordant with the previously cited almost complete absorption of a 200-μg dose of folic acid in ileostomy patients.

Bioavailability

Folic acid pharmacokinetics were determined to be independent of circadian variations.[54] After absorption, folic acid enters the hepatic portal vein predominantly unchanged.[52,55] Doses of up to 260-280 μg are almost completely sequestered by the liver[52] and are subject to first-pass metabolism. Folic acid molecules that enter the liver are either converted into polyglutamate storage forms or metabolized to physiologically active reduced pteroylmonoglutamates, such as L-5-methyltetrahydrofolate (L-5-MTHF).[42,55] These folates may enter the systemic circulation via the hepatic vein or may be excreted into the bile and subsequently reabsorbed in the small intestine, completing enterohepatic recirculation.[43] The liver has a lower affinity for the removal of L-5-MTHF from the portal vein than for folic acid, which allows for the newly formed and reabsorbed L-5-MTHF to directly enter the systemic circulation, where it is the predominant plasma folate under physiological conditions.[43] At doses exceeding ~280 μg folic acid, untransformed folic acid also appears in the plasma.[43,52] Unlike low physiological doses of folic acid, which are almost completely absorbed, higher doses seem to be absorbed incompletely, possibly due to the saturable active uptake mechanism.

The interpretation of the plasma response to an orally administered dose of folic acid yields various difficulties: (1) Single dose administration of folic acid demonstrated linear pharmacokinetics using oral doses of 1.1 and 5 mg, but after repeated administration of 1.1 and 5 mg folic acid, respectively, the steady-state folate concentrations in red blood cells only showed a twofold difference, implying nonlinear pharmacokinetics after repeated administration of high folic acid doses, possibly due to limited uptake mechanisms or low metabolic capacity.[56,57] (2) The plasma response to an oral dose of folic acid does not only originate from the administered dose itself but also consists of L-5-MTHF being displaced from the tissues into the plasma in amounts that can only partly be explained by enterohepatic recirculation of folates stored in the liver.[53] (3) Absolute BA calculated from urinary recovery of isotope-labeled folic acid and metabolites exceeds 100% for orally administered folic acid, indicating different renal elimination kinetics for orally and intravenously administered folic acid.[58] Some suggestions have been made in the literature about how to address these difficulties.[48,53]

Distribution and Metabolism

Folic acid is absorbed without undergoing biotransformation in the mucosal absorptive cells and is subsequently sequestered from the portal vein by the liver, the main initial site of folic acid metabolism.[43] The first metabolic step is the reduction of folic acid in hepatocytes via dihydrofolate reductase to dihydrofolate and subsequently to tetrahydrofolate (THF). Compared to other mammals, humans exhibit reduced and more variable dihydrofolate reductase activity,[43,44] resulting in facile saturation of the conversion of folic acid and thus leading to unchanged folic acid appearing in the plasma.[55] THF is further converted to methylene-THF by serine-hydroxymethyltransferase and reduced to physiologically active L-5-MTHF via methylenetetrahydrofolate reductase (MTHFR).[44,59] MTHFR is subject to genetic polymorphism, and a mutation resulting in low activity of MTHFR involves the risk of elevated homocysteine plasma levels and a higher incidence of fetal neural tube defects during pregnancy.[44]

Folic acid as well as its physiologically active metabolite, L-5-MTHF, can be bound to plasma proteins when entering the systemic circulation. The fraction of folic acid bound to plasma proteins, particularly albumin, is stated in the literature to be ~50%[60] or 64%,[61] depending on the reference considered. The volume of distribution of L-5-MTHF is estimated to be 32.0 L,[62] in accordance with the high affinity of most tissues for folates and the rapid distribution into the cells following an oral dose of folic acid.[61] Intracellular reduced folates are involved in vital metabolic reactions such as the synthesis of purines and thymidylate as well as the metabolic conversion of homocysteine into methionine.[44,63]

Excretion

Folic acid and its degradation products are eliminated via urinary excretion (~62%) and to a lesser extent via feces (~38%).[64] Urinary radioactivity plots show 2 different overlapping elimination kinetics: one representing newly absorbed folates being eliminated with a shorter half-life of 31.5 h and the other one corresponding to the turnover of the folate body pool with a longer biological half-life of ~100 days.[50] Unbound folic acid and other plasma folates are filtered at the glomeruli and can undergo concentration-dependent reabsorption in the proximal tubules. The reabsorption process is mediated by FR_{α} folate receptors expressed at the luminal brush-border membrane and appears to be saturable.[42,61,65] Physiological amounts of folate filtered in the glomeruli are almost completely reabsorbed, with virtually no folate lost via urinary excretion. At higher concentrations, the reabsorption is saturated and unchanged folates are excreted at a rate proportional to the plasma level.[65]

The degradation products of folic acid and its physiologically active metabolites found in urine are para-aminobenzoylglutamate and para-acetamidobenzoylglutamate, which are presumably formed by cleavage of tissue folates.[66,67] At a daily intake of 450-μg folate, the excretion of degradation products was shown to be greater than the excretion of intact folates, whereas at a daily intake of 850-μg folate, the amount of intact folates found in urine exceeded the amount of degradation products.[66] In an absolute BA

study conducted by Menke et al.,[48] it was found that 63.6% of a 5-mg orally administered dose of folic acid was excreted unchanged.

Dosage Form Performance

Excipients

Various excipients are used to manufacture immediate-release folic acid tablets with market authorization in 21 countries of the European Economic Area as well as in the United States. The excipients used in the formulation of these tablets are described in Table 3. Colorants, flavors, water, and substances present in the coating were excluded from this list, as coating substances in IR formulations are not expected to alter the drug's pharmacokinetics. In the third column of this table, the minimum and maximum quantities of these excipients that are found in other marketed products in the US are presented, according to the FDA inactive ingredient database. No studies reporting any influence of the excipients listed in Table 3 on the absorption of folic acid were found in the literature search.

Dissolution

According to the 40th edition of United States Pharmacopoeia (USP 40), the specification for the dissolution test of tablets containing folic acid is Q $\geq$75% of the labeled strength released in 45 min using apparatus 2 with 50 rpm and 500 mL of distilled water as dissolution medium.[68]

To evaluate the quality of products containing folic acid, Younis et al.[69] analyzed the pharmaceutical performance of 15 products available on the American market, taking into account the pharmacopeial specifications regarding disintegration and dissolution. For each tested product, the following media were used: simulated gastric fluid, simulated intestinal fluid, and distilled water using apparatus I for capsules and apparatus II for tablets, along with the test conditions specified for folic acid tablets and folic acid in multivitamin dosage units in the USP 26, which was the current pharmacopoeia at the time of their investigations. On evaluation, 14 of the 15 tested products met the disintegration specifications, disintegrating in less than 30 min. Regarding dissolution, all the tested products met the requirements when the following media were used: distilled water (pH 5-6) and simulated intestinal fluid (pH 7.5), with the total amount released being 85%-143% and 89%-135%, respectively. However, all products failed in simulated gastric fluid (pH 1.2), with the lowest percentage released being 23% and the highest being 68%. The explanation proposed for this incomplete release was the dependency of folic acid solubility on the medium pH. The authors speculated that slow dissolution or disintegration behavior may affect absorption and, consequently, folic acid BA.[69] More recently, Bellavinha et al.[36] published results from a study investigating the pharmaceutical performance of 2 products available on the Brazilian market. Dissolution studies were performed using USP apparatus II (paddle method) with $n = 6$ at 37 $\pm$ 1°C. Three different buffers (pH 1.2, 4.5, and 6.8) were used as the dissolution media (900 mL) and the paddle rotation speed was kept at 50 rpm. In all experiments, at predetermined time intervals (1, 5, 10, 15, 30, 45, 55, 65, 75, 95, and 105 min for media at pH 1.2 or 4.5; and 1, 3, 5, 10, 15, and 30 min for the medium at pH 6.8), the dissolution profiles of the tablets were compared via dissolution efficiency (%), using t-test and analysis of variance. Although the profiles for the products obtained at pH 1.2 were similar ($p > 0.05$), the profiles obtained in media with pH 4.5 and 6.8 were dissimilar ($p < 0.05$). Evaluating the same product in each of the 3 media, the dissolution profiles obtained did not show similarity ($p < 0.05$). None of the products were able to show rapid dissolution in pH 1.2 and 4.5 media, in accordance with the low solubility at low pH values, and therefore, the folic acid products studied could not meet biowaiver requirements for dissolution.

Discussion

Solubility

According to the current regulatory guidelines, an API is considered highly soluble if the dose/solubility ratio (D/S) is below 250 mL at 37°C for the "highest dose strength"[8] or the "highest single therapeutic dose"[6,9] in the pH range of 1-6.8[8] or 1.2-6.8,[6,9] respectively. For the highest dose strength of 5 mg for folic acid, the D/S was found to be higher than the critical limit at pH $\leq$ 4.5 in all solubility studies depicted in the experimental solubility section, except for the study performed by Younis who found the D/S to be below the critical limit at all pH values. The experimental conditions in the solubility study performed by Younis[35] differed from the conditions chosen in the other studies. A water bath was used for sample heating, the samples were stirred without identification of the rotational speed, and the studies were carried out for up to 5 days per sample. These differences may have caused the discrepancies in the solubility values determined at different pH values compared to those of other authors, as shown in Table 2. Taking into account only the more recently performed studies by Wu et al.,[32] Bellavinha et al.,[36] and current results obtained at the Goethe University, Frankfurt, essentially similar pH-dependent solubility profiles for folic acid have been obtained in all 3 studies (Fig. 2). In studies that included samples measured at pH 3 (Wu et al. and current results), a *U*-shaped solubility profile was obtained with the solubility being minimal around pH 3, in accordance with the expected low solubility of predominantly uncharged folic acid at pH values between 2.4 and 3.5, as described in the p*Ka* section.

Based on the recent solubility studies, folic acid can safely be classified as "not highly soluble" because the D/S-ratio is more than 10 times higher than the critical limit of 250 mL at pH 3.

Permeability

According to the recent guidance draft of the FDA,[8] the European Medicines Agency Guidance,[9] and the WHO Guidance,[6] a drug is considered highly permeable when the extent of its absorption is 85% or higher.

On analysis of the data found in literature, there is a consensus regarding almost complete absorption ($\geq$90%) of small, physiological doses of folic acid ($\leq$320 µg).[50-53] At these low doses, the permeability would be almost surely driven by active transport, whereas at a 5-mg dose, active factored is presumably saturated and passive transport must also be factored in. Mass balance studies with folic acid doses lower than 320 µg therefore likely overpredict the fraction absorbed for higher doses.

Based on Caco-2 permeability studies, there is evidence for a classification of the drug as moderately to poorly permeable,[49] but because active transport mechanisms are underexpressed in CaCo-2 cells, the permeability found in these studies might be an underestimate of the effective permeability *in vivo*.

When considering a single dose of 5-mg folic acid, permeability data are inconclusive, as there is no reliable f_{abs} value from mass balance studies for those therapeutic doses reported. Data obtained from a BA study performed in humans with 5 mg of orally administered folic acid indicated that the mean absolute BA is 76.2% with the individual values ranging from 49.3% to 96.7%, meaning that in some subjects almost the complete relative amount of the oral dose was found in the plasma, while in other subjects only half of the amount was found when comparing oral versus i.v. administration.[48] The difference in the absolute BA observed among study

Table 3
Excipients[a] Present in Folic Acid IR Solid Oral Drug Products[b] With a Marketing Authorization (MA) in Austria (AT), Belgium (BE), Czech Republic (CZ), Germany (DE), Denmark (DK), Spain (ES), Finland (FI), France (FR), Greece (GR), Croatia (HR), Hungary (HU), Ireland (IE), Iceland (IS), Italy (IT), The Netherlands (NL), Norway (NO), Portugal (PT), Romania (RO), Sweden (SE), Slovakia (SK), United Kingdom (UK), and the United States (U.S.),[c] and the Minimal and Maximal Amount of that Excipient Present Pro Dosage Unit in Solid Oral Drug Products With an MA in the U.S.[d]

Excipient	Drug Products Containing That Excipient With an MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms With an MA in the US (mg)
Acacia	CZ(1) SK(2) UK(3)	5-156[e]
Alginic acid	FI(4) HU(5,6)	3.6-80
Butylhydroxyanisole	RO(7)	0.07-0.5
Calcium hydrogen phosphate	FI(4) FR(8) HU(5,6) IE(9) SE(10,11)	30-850
Calcium stearate	CZ(1) SK(2)	0.7-43
Carmellose sodium	CZ(1) SK(2)	3.2-160
Cellulose, microcrystalline	BE(12) DE(13) ES(14) FI(15) FR(8,16-18) GR(19) HR(20) HU(5,6) IE(9) IS(21) IT(22-25) NL(26,27) PT(28-30) RO(7) SE(10,11,31) US(32-47)	4.6-1553[e]
Cellulose, powdered	DE(48-55) IE(56) SE(57)	20-560[e]
Crospovidone	ES(58) HR(20) PT(59,60) SE(31) US(32)	4.4-365[e]
Gelatin	CZ(1) DK(61) NO(62) SK(2)	1-733[e]
Lactose	AT(63) BE(12) CZ(1) DE(13,48-55,64) DK(61) ES(14,58,65,66) FI(15) FR(16,18) GR(19,67) HR(20) HU(68) IE(56,69,70) IS(21) IT(22-25,71) NL(26,27,72) NO(62) PT(28-30,59,60) RO(7) SE(31,57) SK(2) UK(3,73,74) US(33-37,39,41-47,75)	23-2217[e]
Macrogols	AT(63) DE(64)	0.13-1057[e]
Magnesium stearate	AT(63) BE(12) DE(13,48-55,64) DK(61) ES(14,58,65,66) FI(4,15) FR(8,16-18) GR(19,67) HR(20) HU(5,6,68) IE(9,56,69,70) IS(21) IT(22-25,71) NL(26,27,72) NO(62) PT(28-30,59,60) RO(7) SE(10,11,31,57) UK(3,74) US(32,34,43,75)	0.15-4384[e]
Magnesium trisilicate	FR(17)	12-77
Povidone	ES(58,65) HR(20) HU(68) IT(71) PT(59,60) RO(7) SE(10,11,31) US(32)	0.17-240
Silica	BE(12) DE(13,48-55) ES(65,66) FI(15) FR(8,16,17) IE(56) IS(21) IT(24,71) NL(26) PT(28,29) SE(57) UK(74) US(32,36,39,47,75)	0.1-138
Sodium starch glycolate	DE(13) ES(14) FI(15) FR(18) GR(19) IT(22,23,25) NL(27) PT(30) SE(10,11) US(33-47)	2-870[e]
Starch	AT(63) CZ(1) DE(64) DK(61) ES(65) FR(16) GR(67) HU(5,6,68) IE(9,69,70) IS(21) IT(24,71) NL(26,27,72) NO(62) PT(28,29) RO(7) SK(2) UK(3,74) US(32,38,40,75)	0.44-616[e]
Starch, pregelatinized	BE(12) DE(13) FR(8,17) HU(5,6) IE(69,70) NL(27,72) UK(73)	4.0-482
Stearic acid	AT(63) DE(64) IE(9) UK(3,73) US(33,35-42,44-47)	0.9-72[e]
Sucrose	AT(63) CZ(1) DE(64) SK(2) UK(73)	0.02-9700[e]
Talc	CZ(1) DE(48-55) DK(61) ES(65,66) HU(68) IE(56) IT(71) NL(72) NO(62) RO(7) SE(57) SK(2)	0.1-321[e]

(1) Acidum Folicum Léčiva, Obalené tablety. (2) Acidum folicum Léčiva 10 mg, obalené tablety. (3) Folic Acid 5 mg Tablets [Wockhardt UK Ltd.]. (4) Folvite 1 mg tabletti. (5) Eurovit Folsav 3 mg tabletta. (6) Huma-Folacid 5 mg tabletta. (7) Acifol 5 mg comprimate filmate. (8) Speciafoldine 0.4/-5 mg cp. (9) Preconceive 400 μg Tablets. (10) Folacin 1/-5 mg tabletter. (11) Folvidon 5 mg tabletter. (12) Folavit 4, 4 mg tabletten. (13) Folverlan® 0.4 mg Tabletten. (14) Zolico 400. (15) Foliver 1 mg tabletti. (16) Acide Folique Arrow 5 mg cp. (17) Acide Folique CCD 0.4/-5 mg cp. (18) Fertifol 400 μg cp. (19) Folidex 400 μικρογραμμαρια δισκια. (20) Folacin 5 mg tablete. (21) Folic Acid 5 mg töflur. (22) BALFOLIC 400 microgrammi compresse. (23) Fertifol 400 microgrammi compresse. (24) Folicom 5 mg compresse. (25) Folidex 400 microgrammi compresse. (26) Foliumzuur Aurobindo 5 mg, tabletten. (27) Foliumzuur Teva 5 mg, tabletten. (28) Ácido Fólico Generis 5 mg comprimidos. (29) Enser 5 mg comprimidos. (30) Folidex 400 microgramas comprimidos. (31) Folsyra Pilum 5 mg tablett. (32) Folic Acid Tablets, USP 1 mg [Mutual Pharmaceutical Company, Inc.]. (33) Folic Acid Tablets, USP 1 mg [Watson Labs]. (34) Folic Acid Tablets, USP 1 mg [AiPing Pharmaceutical Inc.]. (35) Folic Acid Tablets, USP 1 mg [Amneal Pharmaceuticals of New York, LLC]. (36) Folic Acid Tablets, USP 1 mg [Blu Pharmaceuticals, LLC]. (37) Folic Acid Tablets, USP 1 mg [Cadila Pharmaceuticals Ltd.]. (38) Folic Acid Tablets, USP 1 mg [Excellium Pharmaceutical Inc.]. (39) Folic Acid Tablets, USP 1 mg [Exelan Pharmaceuticals, Inc.]. (40) Folic Acid Tablets, USP 1 mg [Leading Pharma, LLC]. (41) Folic Acid Tablets, USP 1 mg [Major Pharmaceuticals]. (42) Folic Acid Tablets, USP 1 mg [Marlex Pharmaceuticals Inc.]. (43) Folic Acid Tablets, USP 1 mg [Method Pharmaceuticals, LLC]. (44) Folic Acid Tablets, USP 1 mg [Mylan Institutional Inc.]. (45) Folic Acid Tablets, USP 1 mg [Qualitest Pharmaceuticals]. (46) Folic Acid Tablets, USP 1 mg [Sunrise Pharmaceutical, Inc.]. (47) Folic Acid Tablets, USP 1 mg [West-ward Pharmaceutical Corp][II]. (48) DreisaFol® 5 mg Tabletten. (49) Folsäure biosyn Tabletten. (50) Folsäure-Hevert® Tabletten. (51) Folsäure Lomapharm 5 mg Tabletten. (52) Folverlan® 5 mg Tabletten. (53) Folsäure AbZ 5 mg Tabletten. (54) Folsäure Heumann 5 mg Tabletten. (55) Folsäure-ratiopharm® 5 mg Tabletten. (56) Folic Acid 5 mg Tablets. (57) Folsyra Evolan 5 mg tablett. (58) Bialfoli 5 mg comprimidos. (59) Folicil, 5 mg, comprimido. (60) Fovital 5 mg comprimido. (61) Folimet (1/-5 mg). (62) Nycoplus Folsyre 0.4 mg tablett. (63) Folsan 0.4/5 mg Tabletten. (64) Folsan® 0.4 mg/-5 mg Tabletten. (65) Acfol 5 mg comprimidos. (66) Ácido Fólico Aspol 10 mg cápsulas duras. (67) Filicine (folic acid 5 mg tablet). (68) Folsav 3 mg tabletta. (69) Clonfolic 0.4 mg Tablets. (70) Folic Acid 400 microgram Tablets. (71) Folifill 5 mg compressa. (72) Foliumzuur TEVA 0.5 mg, tabletten. (73) Folic Acid Tablets BP 5 mg [Intrapharm Laboratories Ltd.]. (74) Folic Acid Tablets BP 5 mg [Actavis UK Ltd.]. (75) Folic Acid Tablets, USP 1 mg [West-ward Pharmaceutical Corporation].

[a] Colorants, flavors, water, and ingredients present in the coating are not included. Substances are excluded if it can be assumed that the constituents are only present in the coating/polish.

[b] Excluded are soft gelatin capsules filled with a solution.

[c] Sources of data: AT, www.basg.gv.at (Accessed April 03, 2017); BE, www.bcfi.be (Accessed April 03, 2017); CZ, www.sukl.cz (Accessed April 03, 2017); DE, www.rote-liste.de; (Accessed April 03, 2017); DK, www.dkma.dk (Accessed April 05, 2017); ES, www.aemps.es (Accessed March 28, 2017); FI, www.fimea.fi (Accessed April 03, 2017); FR, www.vidal.fr; (Accessed April 03, 2017); GR, www.eof.gr (Accessed April 05, 2017); HR, www.almp.hr (Accessed March 29, 2017); HU, www.ogyi.hu (Accessed April 03, 2017); IE, www.hpra.ie (Accessed March 29, 2017); IS, www.serlyfjaskra.is (Accessed March 29, 2017); IT, www.torrinomedica.it (Accessed March 29, 2017); NL, www.cbg-meb.nl. (Accessed April 03, 2017); NO, www.legemiddelverket.no (Accessed March 29, 2017); PT, http://www.infarmed.pt/infomed/ (Accessed April 03, 2017); RO, www.anm.ro (Accessed March 29, 2017); SE, www.lakemedelsverket.se (Accessed March 29, 2017); SK, www.sukl.sk (Accessed March 29, 2017); UK, www.medicines.org.uk/emc (Accessed March 29, 2017); U.S., www.dailymed.nlm.nih.gov (Accessed April 04, 2017).

[d] U.S.: FDA's Inactive Ingredient Database, https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm (version date January 10, 2017).

[e] The upper range value reported is unusually high for solid oral dosage forms and the authors doubt its correctness.

participants could possibly be explained by the aforementioned saturable active uptake mechanism and inefficient passive transport, as well as by sequestration in the liver and saturable, variable first-pass metabolism.

On account of the lack of reliable permeability data for 5 mg doses, folic acid in the highest dose strength is conservatively classified as "not highly permeable."

Biopharmaceutics Classification System

Other research groups have classified folic acid according to the BCS scheme, but in most cases, only a provisional classification was made. Lindenberg et al.[70] classified folic acid as a BCS class II/IV compound, considering the data regarding permeability to be inconclusive. According to WHO data, the literature data regarding

the permeability of folic acid were also found to be inconclusive, with folic acid belonging to BCS class I or III.[7] The solubility data shown in Table 2 combined with the inconclusive permeability data from the literature corroborate the suggestion from Lindenberg et al. that folic acid belongs to BCS class II or IV.

In this biowaiver monograph, as a result of the experiments and literature reviewed, folic acid is provisionally classified as a BCS IV compound on the basis of its observed low solubility at acidic pH values and the permeability data, which suggest incomplete absorption due to saturable active uptake mechanisms at dose strengths of 1-5 mg. Until additional permeability data become available for this dosage strength range, this classification should be used.

Risks of Bioinequivalence Caused by Excipients and Production

No study investigating the influence of the excipients listed in Table 3 on the BA of folic acid in oral formulations has been reported in the open literature. Generally, when BE demonstration with a reference product is sought, the formulation of the test product should contain excipients that are well established for solid oral dosage forms and should be present in quantities that do not exceed the usual quantities used in the respective immediate-release dosage forms. As indicated in the last column of Table 3, all excipients used in manufacturing folic acid drug products are well established because they are commonly used in other immediate-release drug products and listed on the FDA inactive ingredient database.[71] Except for macrogol, no "critical excipients" that could possibly interfere with the absorption of folic acid or alter the gastrointestinal transit time was found. Macrogol is listed as an excipient in a folic acid formulation with MA in Germany and Austria (Folsan® 0.4 mg/-5 mg Tabletten) and it could be assumed to have a potential effect on BA because high concentrations of macrogols act as laxatives and might shorten gastrointestinal transit time. The macrogol type used as an excipient in Folsan is macrogol 4000, and as a laxative, the usual dose is 10 g dissolved in a glass of water (~250 mL), taken orally 2 times a day.[72] Because the average tablet weight of Folsan tablets is only about 120 mg, the resulting concentration of macrogol 4000 after the intake of Folsan is unlikely to influence the gastrointestinal transit time.

Although folic acid is not a highly soluble drug and therefore a potential effect of differences in the manufacturing process on absorption cannot be ruled out completely, the excipients commonly used for formulation of immediate-release solid oral dosage forms containing folic acid are unlikely to pose a risk of bioinequivalence if 2 products are manufactured with different excipients.

Regarding the selection of comparator products to be used in BE testing, the WHO guidance document "Guidance on the selection of comparator pharmaceutical products for equivalence assessment of interchangeable multisource (generic) products"[73] should be followed.

There is no longer a folic acid innovator drug product available on the U.S. market (Folvite 1 mg was the first folic acid drug product in the United States, with market approval being granted in 1947). Thus, following the general principles stated in the WHO Guidance, a national market leader product for which a national marketing authorization has been granted or a product that has been granted approval in an International Conference on Harmonisation–associated country may be selected as a comparator product for BE trials when no innovator product is available.

Patient's Risks Associated With Bioinequivalence

When a biowaiver procedure for a drug is considered, its safety limits must be taken into account.[7,8] The therapeutic indications of folic acid are limited to prevention and treatment of vitamin B_9 deficiency and hyperhomocysteinemia, as well as prophylactic supplementation during pregnancy aiming to avoid malformation of the neural tube. Folic acid is considered a safe drug when it is used according to a precise diagnosis and with respect to the general principles of therapy.[1] As described in the Therapeutic Index and Toxicity section described previously, there are no substantiated reports of severe acute adverse effects regarding the intake of 5 mg folic acid. There is, however, still uncertainty in the literature concerning possible adverse effects of long-term supplemental folic acid intake that have yet to be investigated in larger randomized clinical trials. Incorrect biowaiver decisions (e.g., incorrectly granting supra-/sub-bioavailable products market authorization) would unlikely have a negative effect on the treatment. Because folic acid is not physiologically active until metabolized, and large doses of folic acid are eliminated unchanged, the therapeutic effect is solely limited by the metabolic capacity that would be easily saturated even with sub-bioequivalent formulations. In this context, where the existence of a wide therapeutic window for the short-term treatment with therapeutic doses of folic acid can be inferred, an incorrect biowaiver decision would therefore be unlikely to result in serious problems for public health.

As an example of recognition of lack of patient risk, the German legislation allows for a market authorization of specified drug products in the form of "approval exempt standard formulations" based on monographs approved by the national department of health, without the need for either *in vivo* or *in vitro* BE testing. As a prerequisite for this process, there must not be any expectation of a risk to the public health.[74] To gain approval, the drug product must have the composition and be manufactured according to an official monograph. For folic acid, such a monograph exists for approval of tablets containing 5 mg folic acid (approval number 1909.99.99).[75] It can therefore be concluded that the national health authorities in Germany perceive that there is no risk to public health associated with generic folic acid formulations in dose strengths of 5 mg manufactured according to this monograph.

Conclusion

Because folic acid is clearly not highly soluble over the required pH range, it can be concluded that it belongs to either BCS class II or IV. However, the permeability and fraction absorbed data found in the literature are not conclusive for high (5 mg) doses, so it is difficult to reliably classify folic acid within the BCS criteria. Conservatively, a provisional classification of BCS IV is assigned. Furthermore, none of the evaluated folic acid formulations has shown rapid dissolution in media at pH 1.2 or 4.5 in dissolution studies published by Bellavinha et al.[36] In this context, the biowaiver approach for folic acid in immediate-release solid oral dosage forms cannot be recommended according to the current BCS Biowaiver Guidances of the United States, Europe, and WHO. However, we note that at least the German regulatory authority considers folic acid 5 mg tablets to present such a low risk to public health that they are allowed to be marketed without either *in vitro* or *in vivo* BE studies, as long as the composition and manufacturing procedure comply with the official monograph.

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Global Health Commentary

The Discriminatory Power of the BCS-Based Biowaiver: A Retrospective With Focus on Essential Medicines

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ABSTRACT

This article summarizes historic developments, recent expert opinions, and (currently) unresolved challenges concerning the Biopharmaceutics Classification System (BCS)-based Biowaiver. An overview of approval statistics and application potential, case examples addressing the discriminatory power of the procedure, as well as an outlook on possible refinements in the future are provided and critically discussed. Over the last decade, regulatory guidance documents have been harmonized, for example, following scientific consent on allowing biowaivers for BCS class III drugs, making over 50% of orally administered drugs on the World Health Organization Essential Medicines List eligible for an abbreviated approval. Biowaiver monographs that present a complete risk-benefit evaluation for individual drugs have been issued by the International Pharmaceutical Federation for more than 25% of those drugs with the long-range aim of covering all essential drugs. Unresolved issues that have emerged from reported examples of false-negative and false-positive outcomes in the literature demand further adjustments to the regulatory requirements. Possible solutions for resolving these issues are the use of modeling and simulation and refined biorelevant *in vitro* tests that are better able to discriminate between dosage forms with unequal performance *in vivo*, potentially allowing biowaivers for selected BCS II drugs.

Introduction

When the Biopharmaceutics Classification System (BCS) was established by Amidon et al.[1] in 1995, a sound regulatory basis for assessing possible bioavailability (BA) problems of drugs formulated as solid oral dosage forms was created by assigning active pharmaceutical ingredients (APIs) to 1 of 4 BCS classes based on their biopharmaceutic properties. Originating from this system, the approval of generic drug products that yield a low risk of BA problems was made possible, based solely on *in vitro* dosage form performance comparison instead of *in vivo* pharmacokinetic bioequivalence (BE) studies. The Food and Drug Administration (FDA) in the United States was the first regulatory authority to draft a guidance document on waiving the requirement for *in vivo* BA and BE studies for the approval of generic drugs, introducing the BCS-based Biowaiver procedure for BCS class I drugs in 2000.[2] Other international agencies and regulatory authorities such as the World Health Organization (WHO) and the European Medicines Agency (EMA) followed the FDA approach, introducing their own guidance documents in 2002[3] (EMA) and 2006[4] (WHO) (Fig. 1a). Those first guidance documents differed significantly from each other, for example, in the cutoff value for high permeability (90% in the FDA guidance, 85% in the others) and the pH range to be considered for high solubility. While FDA and EMA only allowed BCS class I drugs for a BCS-based Biowaiver, the WHO guidance also allowed BCS class III as well as some weakly acidic, highly permeable drugs from BCS class II.

Over the last 2 decades, a steadily increasing number of scientific publications on the BCS and the BCS-based Biowaiver has appeared in the literature (Fig. 1a). As well as impacting other research areas, they led to a revision of the aforementioned regulatory guidance documents. Efforts to harmonize the various guidelines have also been made and led to the recent publication of the ICH M9 draft guideline that is currently (May 2019) under public consultation. However, although many publications contributed to an extension of the procedure to BCS class III drugs, there were also publications that raised concerns, especially with respect to waiving BE studies for BCS class II drugs, excipient effects on the BA of BCS class III drugs and the overall discriminatory power of the procedure.

Conflicts of interest: The authors declare none.
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0022-3549/

The purpose of this article is to further elucidate the role of the BCS-based Biowaiver in drug approvals, to summarize common issues, and to provide an overview of the current regulatory and scientific status. The applicability of the procedure for generic drug products containing APIs listed on the 20th WHO Essential Medicines List[6] (EML) is examined to assess the potential number of essential drugs that could be made accessible via abbreviated approvals for market authorization. Recent publications concerned with failures of the procedure (either false-positives or false-negatives) are reviewed in detail to assess the discriminatory power. Finally, controversial aspects, possible solutions and room for extension of the current criteria to other BCS classes found in the pharmaceutical literature are critically discussed.

Methods

Publicly available databases from regulatory authorities were screened for data on drug approvals via BCS-based Biowaiver, including FDA[7] and EMA[8] product-specific guidance documents for generic drug development, as well as EMA.[9] Heads of Medicines Agencies,[10] and Medicines and Healthcare Products Regulatory Agency[11] public assessment reports (PARs). Current guidance documents on waiving *in vivo* BE studies issued by WHO, FDA, and EMA were summarized and compared, and recent publications addressing the BCS-based Biowaiver in the pharmaceutical literature were reviewed via PubMed (https://www.ncbi.nlm.nih.gov/pubmed/) using the search term "Biowaiv*[Tiab]" to compile recent scientific developments and opinions.

In addition, literature data on solubility and solubility classification of drugs on the 20th WHO EML[6] formulated as immediate-release, orally administered solid oral dosage forms were obtained searching for the drug name combined with "solubility" or "BCS class*" on PubMed to obtain an overview of drugs that could potentially benefit from an approval via the BCS-based Biowaiver.

Dissolution tests performed at the Institute of Pharmaceutical Technology, Goethe University Frankfurt am Main, over the last years that met the conditions specified in the regulatory guidance documents were examined to locate dosage form performance failures of products containing drugs otherwise eligible for an approval via the biowaiver procedure.

Results and Discussion

Comparison of Current Guidance Documents

An overview of the current, revised guidelines is given in Table 1. The various guidance documents have already harmonized most of the former discrepant criteria following growing scientific consensus, allowing BCS-based Biowaivers for drugs that belong to class I or III, with similar criteria for permeability (fraction absorbed [f_a] $\geq$ 85%) and solubility classification (D/S $\leq$ 250 mL over the physiological pH range). Differences that still exist among the guidelines are for example the dose to be considered (highest dose strength vs. highest single dose), the recommended dissolution media volume (500 mL vs. 900 mL), and rotational speed (50 or 75 rpm), which supportive permeability data (*in vivo* intestinal perfusion in humans, animal data or *in vitro* cell culture data) are acceptable as well as allowable excipient variations. There is a general consensus in the scientific literature about the necessity to harmonize those remaining ambiguities[17-19] and the International Committee on Harmonization has already embarked on this course and drafted a harmonized guidance that is expected to be finalized in 2019.

Dose Definition

The discrepant definitions of the dose that is to be used for solubility determinations can have a large impact on the solubility classification of an API, and may cause an API to have different BCS classes based on the dose considered. When changing from the FDA definition of "highest dosage strength" to the WHO and EMA definitions of "highest single dose", some APIs would be reassigned from BCS classes I/III into II/IV, thus precluding them from a possible BCS-based Biowaiver. Sediq et al.[20] evaluated the impact of a possible BCS shift for all APIs for which a biowaiver monograph had been issued before 2014. They found that the former classifications were no longer valid for metoclopramide and verapamil, and should be re-evaluated for prednisolone and prednisone. The difference between the 2 dose definitions ranged from no change (highest single dose = highest dosage form strength) to a 5-fold difference (highest single dose = highest dosage form strength × 5), with a median 2-fold increase. To address the problem of

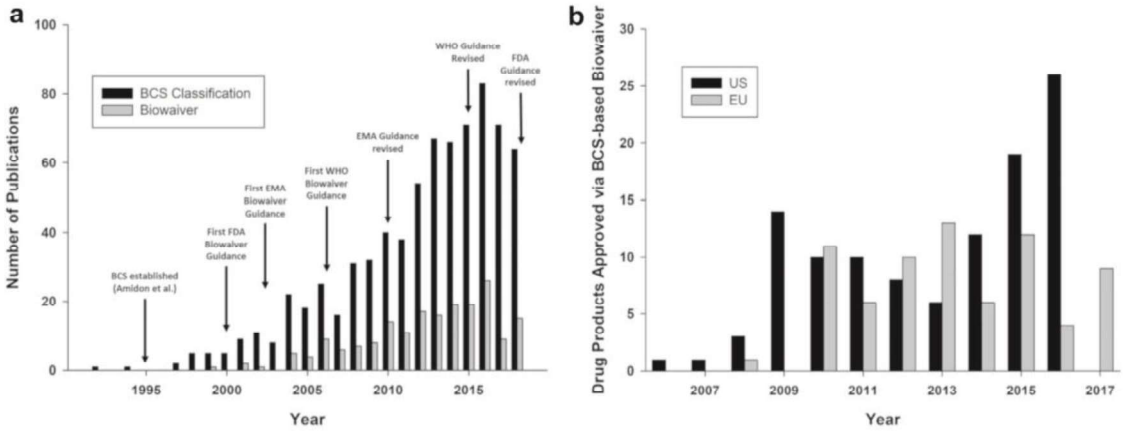

Figure 1. (a) Number of publications per year using the keywords "BCS Classification" or "Biowaiver", annotated with historic regulatory developments. (b) Number of FDA-approved abbreviated new drug applications (ANDAs) via BCS I Biowaiver (adopted from Mehta et al.[5]) and approved BCS-based Biowaivers in the EU per year based on public assessment reports and annual reports (last accessed: November 2018).

Table 1
Comparison of the WHO, FDA, and EMA Biowaiver Guidance Documents (a More Detailed Version Can Be Found in the Supplementary Material)

Parameters	WHO (2015)[12]	FDA (2017)[13]	EMA (2010)[14]
Solubility			
Method	Shake-flask or other justified method	$n \geq 3$; shake-flask method or other (e.g, titration); USP buffers favorable; pH verification before and after API addition and at the end; validated stability-indicating assay; degradation should be reported	Replicate determinations; shake-flask or other justified method; solution pH should be verified before and after addition of the drug substance to a buffer
Temperature	37 ± 1°C	37 ± 1°C	37 ± 1°C
pH	1.2-6.8	1.0-6.8; sufficient number of pH conditions, that is: pH = 1, 6.8, pK_a, pK_a+1, pK_a-1	≥3 buffers (preferred pH 1.2, 4.5, 6.8 and pK_a if within range)
Dose strength	Highest single therapeutic dose	Highest strength of an IR product (if single dose is higher: additional information needed)	Highest single therapeutic dose
Highly soluble specification	D/S ≤ 250 mL	D/S ≤ 250 mL	D/S ≤ 250 mL
Permeability			
Highly permeable specification	$f_a \geq 85\%$ in mass balance determination or absolute BA studies (same dose as in solubility studies or proof of dose linearity)	$f_a \geq 85\%$ in mass balance determination, absolute BA studies or human intestinal perfusion; single method sufficient when: $BA_{abs} \geq 85\%$, ≥85% recovered unchanged in urine or ≥85% recovered parent and metabolites in urine and proof of stability in GI tract	$f_a \geq 85\%$ in mass balance determination or absolute BA studies; metabolites formed after absorption (phase 1 & 2) should be taken into account (urinary and fecal recovery), but not degradation products formed in the GI tract
Other acceptable data	*in vivo* intestinal perfusion in humans with reference compounds (permeability >85%) and negative control	For example, *in vivo* or *in situ* animal intestinal perfusion, *in vitro* epithelial cell culture methods or excised intestinal tissues	None stated
Supportive data	*in vivo* or *in situ* animal intestinal perfusion; *in vitro* epithelial cell culture methods (validated using APIs with known permeability)	Evidence showing stability of the drug in the GI tract is needed to support mass balance studies	Reported BE between aqueous and solid formulations; well performed *in vitro* permeability investigations including reference standards
BCS classification			
Eligible BCS class	BCS I and BCS III	BCS I and BCS III	BCS I and BCS III
Dissolution			
Apparatus	USP II (paddle) or USP I (basket)	USP II (paddle) or USP I (basket)	USP II (paddle) or USP I (basket)
Agitation speed	75 rpm (paddle) 100 rpm (basket)	50 rpm or 75 when justified (paddle), 100 rpm (basket)	50 rpm (paddle), 100 rpm (basket)
Dissolution media	pH 1.2: HCl solution or buffer; pH 4.5: acetate buffer; pH 6.8: phosphate buffer; pharmacopoeial buffers recommended; no surfactants; enzymes may be used if gelatin is involved	0,1 N HCl or SGF_{sp} (USP); pH 4.5 buffer; pH 6.8 buffer or SIF_{sp} (USP)	pH 1-6.8 (at least pH 1.2, 4.5, 6.8): 0,1 N HCl or SGF_{sp}, pH 4.5 buffer, pH 6.8 buffer or SIF_{sp}; Ph. Eur. buffers recommended; additional pH with minimum API solubility; no surfactants; enzymes may be used if gelatin is involved; pH should be ensured throughout experiment
Temperature	37 ± 1°C	37 ± 0.5°C (refers to USP chapter <711 Dissolution>)	37 ± 1°C
Volume	≤900 mL	≤500 mL (≤900 mL when justified)	≤900 mL
Sample size	12 samples (for f_2 testing)	12 samples	12 samples, more than 1 batch
Sampling	For example, 5, 10, 15, 20, 30, 45, 60 min	Sufficient number, for example, 5, 10, 15, 20 and 30 min	For example, 10, 15, 20, 30 and 45 min
f_2-test	Comparison of mean dissolution values; CV ≤ 20% until 10 min, ≤10% afterward; ≥3 time points (zero excluded and same for both formulations); only 1 time point after comparator releases 85%; surfactants should be avoided	Comparison of mean dissolution values; CV ≤ 20% at earlier time points (up to 15 min), ≤10% afterward; only one time point after 85% release	Comparison at 15 min is essential; minimum of 3 time points (zero excluded): before 15 min, at 15 min, release close to 85%, same time points for both formulations, $n = 12$, not more than 1 time point after 85% dissolution, CV ≤ 20% for the first point and ≤10% from second to last time point
Specifications	Very rapidly dissolving (BCS I/III): ≥85% release in 15 min; Rapidly dissolving (BCS I): ≥85% in 30 min and $f_2 \geq 50$		
Excipients			
General Specifications	Well established excipients in usual amounts, no influence on absorption, no PK interactions; Critical excipients (e.g., mannitol, sorbitol, surfactants) should not differ qualitatively and have to be quantitatively similar; BCS I: recommended that excipients are present in the comparator product or in other marketed products; BCS III: excipients should be qualitatively the same and quantitatively similar to the comparator (except colorants, flavors, preservatives without effect on BA); excipient quantity should be consistent with the intended function, atypically large amounts have to be justified		
Additional Specifications	See WHO quality limits on allowable quantitative changes in excipients[15]	Allowed changes in excipients: change in technical grade of excipient, filler ±10%, disintegrant (starch) ± 6%, other disintegrants ±2%, binder ±1%, lubricant (Ca/Mg-stearate) ±0.5%, other lubricants ±2%, glidant (talc) ±2%, other glidants ±0.2%, film coatings ±2%, total change ±10%	All possible excipient interactions affecting BE, solubility, permeability, motility or transporters should be discussed. Regarding allowed changes, EMA refers to the FDA SUPAC guidelines (as stated in a PAR[16])
Risk-benefit analysis			

Considerations	Favorable risk-benefit analysis mandatory; BCS III: extent, site and mechanism of absorption should be critically evaluated; risk review of an incorrect decision (possible influence on therapeutic efficacy and toxicity)	Not stated	Risk of an incorrect biowaiver decision should be more critically reviewed (e.g., site-specific absorption, risk for transport protein interactions, excipient compositions and therapeutic risks) for BCS III compounds than for BCS I compounds
Restrictions			
	No NTI drugs	No NTI drugs; no products designed to be absorbed in the oral cavity	No different API forms (except BCS I salt forms with similar physicochemical properties); no NTI drugs; no sublingual, buccal, and MR formulations
Additional comments			
	Biowaiver approval of proportional formulations, delayed and extended release formulations is possible; *in vitro* equivalence testing for SUPAC may be possible	Legal Basis: Regulations in 21 CFR 320 and 320.22, valid for orally administered INDs, NDAs and ANDAs; prodrugs: permeability measurement of parent or metabolite dependent on site of conversion, dissolution and solubility of both may be relevant; FDC biowaiver is possible	Legal basis: Directive 2001/83 EC; waiver of additional strengths, ODTs, oral solutions and FDCs is possible; waiver for other formulations (liposomal, micellar, emulsions) may be possible under certain conditions

ANDA, abbreviated new drug application; BA, bioavailability; CFR, code of federal regulations; CV, coefficient of variation; D/S, dose/solubility-ratio; f_a, fraction absorbed; FDC, fixed-dose combination; GI, gastrointestinal; IR, immediate release; IND, investigational new drug; MR, modified release; NDA, new drug application; NTI, narrow therapeutic index; ODT, orally disintegrating tablet; PAR, public assessment report; PK, pharmacokinetic; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SUPAC, scale-up and postapproval changes; USP, United States pharmacopoeia.

different BCS classifications based on the dose used, Charkoftaki et al.[21] proposed a dose-dependent BCS system, classifying drugs based on fraction absorbed (f_a), their dose, and a critical dose number to define clearer boundaries for possible biowaiver candidates.

Regarding dissolution testing, use of the "highest single dose" criterion is questionable. Because the BCS-based Biowaiver is a substitute for PK-BE, a case could be made for harmonizing by using the highest dosage strength for the procedure since PK-BE is standardized to highest dose strength. Moreover, although "highest single dose" reflects the maximum dose of a drug product used by patients in everyday life, and thus can be viewed as a "worst case" in terms of discriminating power, there are no experimental data in the literature which demonstrate that using the highest single dose is more discriminating than using the highest dosage strength. Some drugs, for example, corticosteroids, are also rarely given at the highest recommended single dose, and in these cases, using the highest single dose in BE studies or substitutes thereof do not reflect typical practice. Furthermore, there are methodological drawbacks to testing multiple dosage forms in one single vessel. First, changes in hydrodynamics (e.g., higher risk of "coning") and media pH are more likely, thereby increasing variability in the results. Second, because dissolution profile comparison would be generated from multiple dosage forms, a one-to-one comparison of the individual dosage form performances is no longer possible.

Harmonization of the guidance criteria will help prevent the aforementioned inconsistencies in BCS classifications at an international level.

Degradation

All of the guidance documents describe the actions to be taken when an API shows degradation during solubility or dissolution studies rather vaguely. In the guidance sections covering solubility, it is stated that observed degradation should be reported to the regulatory authority, but no additional advice is given. Furthermore, only the FDA guidance[13] describes additional experiments to characterize instability and these are found in the permeability section: when the drug is expected to be unstable in the gastrointestinal fluids, degradation is to be documented in simulated gastric and intestinal fluids or other biorelevant media. A temperature of 37°C and a duration of 1 h for testing in gastric fluid or 3 h when testing in intestinal fluid are proposed. Plöger et al.[22] proposed that stability-indicating dissolution testing as described in the biowaiver monograph for acetylsalicylic acid[23] is a feasible approach to conducting degradation studies. Degradation of 15% is suggested as the cutoff value for significant degradation because greater degradation would result in less than 85% of the API being available for absorption, and thus result in a change in the permeability classification to "not highly permeable". Currently, the FDA takes a more conservative approach, with a cutoff value of 5% for significant degradation.[13]

Permeability Classification

Although the criterion for "high permeability" is now harmonized among the various guidance documents (fraction absorbed [f_a] $\geq$ 85%, Table 1), the methods which can be used vary slightly among them. Uncertainty in the permeability classification is the main reason for most unclear BCS classifications,[24] which is mainly based on the difficulty to directly assess the f_a. The most reliable methods are either absolute BA studies, when it can be demonstrated that the BA is $\geq$85%, or mass balance studies with, for example, radioactively labeled APIs, that allow calculation of the fraction of drug that is absorbed and then eliminated via urine or

excreted in the feces. Those methods are uniformly accepted by all authorities, but in the case of mass balance, studies are laborious and costly to perform. When other methods for permeability determination are used, the FDA advises that 2 different methods be used to support the permeability classification. These other methods include human intestinal perfusion studies, which allow assessing the fraction absorbed in perfused intestinal segment (WHO and FDA) and animal intestinal perfusion studies, which are accepted by the FDA but are only regarded as supportive data by the WHO. The FDA is also the only authority that currently accepts data from *in vitro* methods such as permeability assays using cultured monolayers of epithelial cells (e.g., CaCo-2), in combination with another method. The *in vitro* permeability classification using cultured cell monolayers is only deemed suitable for passively transported APIs. To avoid misclassification because of efflux transporter effects, their expression has to be characterized using reference compounds.

Compared to the WHO and EMA guidance documents, the revised FDA guidance document gives clearer advice on how to assess the permeability, and allows more methods to establish the permeability classification. Harmonization of the other 2 guidance documents at the ICH level would be much appreciated, especially because the EMA guidance is vague in the permeability section with respect to other suitable methods apart from mass balance studies and BA studies. This is of importance because in the case of unclear permeability classification, a "worst-case" approach is taken, resulting in many borderline BCS I/III drugs being handled as BCS class III compounds, meaning that stricter criteria are imposed. To assist in achieving more certain permeability classification, Benet et al.[25] proposed to additionally allow fraction metabolized ≥90%, as described in the biopharmaceutics drug disposition classification system,[26] as a criterion for high permeability because phase I oxidative and phase II conjugative metabolism can only occur after absorption. For many drugs, characterizing metabolization is more facile than characterizing permeability. Supporting their proposal, the BCS and the biopharmaceutics drug disposition classification system overlap in ~90% of the cases,[27] which confirms that this would be a reasonable and safe addition to the guidance criteria for permeability.

Excipients

Allowable excipient variations for formulations containing BCS III APIs are defined differently by the WHO and FDA. The FDA applies the criteria of the "scale-up and postapproval changes" guidance, while the WHO refers to their "guidelines on variations to a prequalified product", where the allowed changes stated in Annex 2 are exactly half the size of the allowed changes in the FDA guidance document.

In the scientific literature, the topic of allowable excipients and changes in their quantities is controversially discussed. It is widely accepted that the permeability-limited absorption of BCS III APIs can be significantly influenced by certain critical excipients such as mannitol, sorbitol, sodium lauryl sulfate (SLS) or Tween 80® by either shortening the intestinal transit time, improving the transcellular permeability, inhibiting efflux transport, or increasing the API's solubility. However, the exact influence of minor quantitative changes in these critical excipients is unclear, as is the influence of qualitative and quantitative changes of other (traditionally regarded as "inactive") excipients such as fillers, binders, and disintegrants. A number of reviews and studies have addressed these issues: Zhang et al.[28] compiled information on 60 excipients that affect drug metabolism, whereas Elder et al.[29] reviewed quality aspects, influences of excipients on BE, and *in vitro* permeability studies, as well as the resulting regulatory implications. To assess possible influences of excipients on permeability, excipients are frequently studied in CaCo-2 permeability assays: Rege et al.[30] investigated the influence of 9 commonly used excipients, and found that SLS, Tween 80®, and docusate sodium could increase the permeability of some APIs, whereas others such as lactose monohydrate, hydroxypropyl methylcellulose (HPMC), or propylene glycol showed no effect. The authors also highlighted difficulties in translating observed differences *in vitro* to the situation *in vivo* because of the high variability in CaCo-2 assays (usually >10%), which makes observing minor changes in permeability difficult, and also because, in some cases, observed large differences *in vitro* do not appear to translate into an impact *in vivo*. Other recent studies also compared results from *in vitro* experiments to *in vivo* animal or human studies. Parr et al.[31] studied the influence of lactose, HPMC, PEG-400, povidone, and SLS in CaCo-2 cells as well as in rat intestinal perfusion studies and found that only SLS in concentrations >0.1 mg/mL led to a noticeable difference in the permeability of several BCS III compounds. Vaithianathan et al.[32] investigated the influence of 14 commonly used excipients in human BE studies using BCS class III model drugs acyclovir and cimetidine. They found an effect on drug absorption only for HPMC, sorbitol, and microcrystalline cellulose (which was confounded with HPMC because it was used in the same formulation). For the other 12 excipients investigated, they concluded that, in contrast to the excipient criteria in the guidance documents, those excipients do not have to be qualitatively the same nor quantitatively similar for BCS class III drugs. The quantities used should simply not exceed the amounts tested in their studies.

Although general recommendations and excipient cutoff values such as the ones determined by Vaithianathan et al.[32] that were found safe in human trials are very valuable, there is still the need for more research regarding *in vivo* excipient effects on the absorption of specific APIs. Some excipients might affect certain APIs more than others, for example, it was demonstrated that sodium bicarbonate can lead to an increase in the peak plasma concentration and an earlier t_{max} of paracetamol, while lactose and other reducing sugars were found to be incompatible with isoniazid,[33] but had no influence on other tested APIs.[31] In a retrospective, top-down approach evaluating former BE trials, Kubbinga et al.[34] also found that the risk of an effect of lactose on drug absorption may vary based on the respective BCS classification of the investigated drug. García-Arieta[35] opined that biowaiver decisions, especially for BCS class III drugs, should be assessed more cautiously, as generalizing excipient effects may be inappropriate for individual APIs. One possible approach to investigating various excipient effects on specific APIs is physiologically based pharmacokinetic (PBPK) modeling. Chow et al.[36] used this approach to simulate changes, for example, in solubility, passive permeability, intestinal metabolism, and transporter activity of specific APIs to identify the parameters most prone to leading to a change in BA.

Further highlighting the need for more research in this field, for 2 of 4 PARs in the European region on BCS III APIs, a BCS-based Biowaiver application was rejected solely because of qualitative or quantitative differences of excipients used in formulations containing BCS III APIs.[16,37]

Choice of Comparator Drug Products

Both PK-BE studies and the BCS-based Biowaiver assess BE using comparator drug products. While in the case of PK-BE studies, possible differences in drug product performance may be masked by either physiological (e.g., slow absorption resulting in a late t_{max}) or pharmacokinetic aspects (e.g., long elimination half-life), the drug product performance is the core aspect assessed in the BCS-based Biowaiver. In addition to being a surrogate for PK-BE, the

BCS-based Biowaiver can therefore also serve as tool for quality control. If an internationally acknowledged comparator list were to exist for each API eligible for the BCS-based Biowaiver, similar performance of generic medicines around the globe could be ensured. However, different comparator products are currently proposed for BE testing among the various authorities. The FDA suggests using products listed in the Orange Book,[38] the WHO refers to their list of prequalified finished pharmaceutical products[39,40] and the EMA allows reference products that have been granted a market authorization in the EU.[14] The need for a harmonized comparator list is not only a theoretical issue emerging from different regulatory proposals, but also of practical concern, as demonstrated by Reddy et al.[41,42] and Löbenberg et al.[43] They compared generic drug products from different countries that contain the same BCS I and III APIs and found that many of them show different dissolution behaviors, with some products complying with the regulatory BCS-based Biowaiver documents and others failing to meet the criteria. Not only did products from various manufacturers differ in their dissolution profiles. Surprisingly, this was also true for generic drug products that are marketed by the same pharmaceutical company, but for different countries.[43] The company explained the observed differences with the need to tailor the generic drug product to the respective local comparator.

The discrepancies among the comparators proposed by authorities and the differences in generics from different countries call for an international harmonization of comparator products to guarantee equal performance of generic drug products, irrespective of the region where the medicinal products are applied. A feasible approach for harmonizing comparator products has been proposed and discussed in detail by Gwaza et al.[44] They suggest that the innovator product from well-regulated markets should be used as the comparator product, not only in BE trials but also for BCS-based Biowaivers.

Harmonization of the Guidance Documents

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) issued a draft guidance document in June 2018 that aims to harmonize the requirements for a BCS-based Biowaiver on an international level.[45] The guidance is closely aligned with the recently revised FDA biowaiver guidance and thus shares most of the FDA solubility and dissolution specifications, proposed methods for permeability determination and allowed excipient deviations, but also adds new aspects that are not found in any of the other major biowaiver guidance documents: in addition to solid dosage forms, the ICH draft considers suspensions eligible for a BCS-based Biowaiver, although it does not yet contain supportive information regarding necessary adjustments to the dissolution method and excipient variations. Permeability classification can be established solely based on data from *in vitro* epithelial cell culture methods, without the need to combine the results with a second *in vitro* method (as required in the FDA guidance when permeability classification is not based on *in vivo* data). Compared to the current FDA guidance document, the cutoff value for significant degradation was increased from 5% to 10% for permeability and solubility classification, but without stating a specific time interval for evaluating the occurrence of degradation in solubility studies. A similar approach to the one taken for degradation during permeability classification (1 h in media simulating gastric environment, 3 h in media simulating intestinal environments) with a cutoff value of $\geq$15% for significant degradation would enable degradation specifications to be aligned with the current permeability specifications (fraction absorbed $\geq$85% for highly permeable drugs). Regarding dissolution testing, the use of purified water is proposed as an alternative to buffers. This addition was likely made to include test conditions required by the Japanese regulatory authority, in which purified water is frequently used in quality control dissolution tests to simulate achlorhydric gastric conditions. Although the aforementioned reason to include purified water as a dissolution media is understandable, it bears certain challenges: purified water lacks any buffer capacity and therefore introduces a major source of variability to dissolution testing due to unstable pH conditions.

Besides the changes that either facilitate the biowaiver procedure or make it more flexible, there are also some changes that complicate the approval via BCS-based Biowaiver when compared with the other guidance documents: dissolution at media pH where the API is least soluble may be required, comparison with a drug product containing a different salt form of the same API is not considered eligible even when the solubility of both salt forms is high (this is allowed, e.g., in the EMA guidance document) and, in case of multiple dosage form strengths, comparative dissolution testing of every strength of the test product with the respective strength of a comparator product is required. The last point is of special concern because this creates a discrepancy between the procedure of the BCS-based Biowaiver and the waiver of additional strengths, where (typically) the highest dosage form strength of a test product is compared to a reference product *in vivo*, and similarity of the other strengths is demonstrated by comparing their dissolution profile with the test product (not the reference product!) used in the BE study. In addition to these questionable alterations, there are also several aspects that are unspecified or are lacking in the current state of the draft document: no definition of "narrow therapeutic index drug" is given, the use of modified vessels such as Peak™ vessels that reduce the occurrence of coning and thus allow for lower, more discriminative rotational speeds with the USP II apparatus is not considered and no suggestions for sampling time points are given.

It is to be hoped that many of the aforementioned issues will be resolved in the finalized document to ensure that the new, internationally harmonized BCS-based Biowaiver guidance is not just a slightly modified version of the FDA biowaiver guidance, but a more refined guidance that compiles the advantages of all current regulatory guidance documents, overcomes ambiguities and inaccuracies and still leaves room for enough flexibility in the procedure to comply with the regulatory standards.

Role of the BCS-Based Biowaiver in Drug Approval—United States and Europe

In line with the continuous refinement of the various guidelines and the scientific consensus confirming the validity of the procedure, the approval numbers have increased over the last decades (Fig. 1b). In the United States, 110 abbreviated new drug application approvals via BCS-based Biowaiver were granted by the FDA since the first FDA biowaiver guidance was published, with a noticeable increase over the last few years.[5] In Europe, the first biowaiver approval as stated in the literature was 2002.[46] However, detailed publicly available data for drug approvals via BCS-based Biowaiver in Europe is sparse. Case examples of biowaiver approvals in Europe can primarily be found by searching in PARs of the EMA,[9] Heads of Medicines Agencies,[10] and the Medicines and Healthcare Products Regulatory Agency.[11] A comparison of the FDA-approved BCS-based Biowaivers and BCS-based Biowaivers granted in the European Economic Area based on data from PARs is shown in Figure 1b. A list of all European PARs mentioning approval via BCS-based Biowaiver can be found in the supplementary material.

Figure 2 depicts the top 4 therapeutic classes of drugs in medicines approved by FDA[5] and in Europe along with the top 4 therapeutic classes of highly soluble APIs listed on the 20th WHO EML.[6]

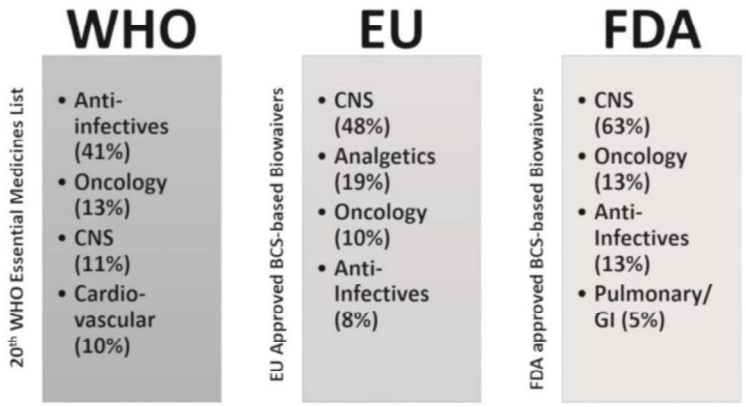

Figure 2. Top 4 therapeutic classes of highly soluble APIs on the 20th WHO EML and among drug products approved via BCS-based Biowaivers by FDA[5] and in the EU.

Most drug products approved via BCS-based Biowaiver are either used to treat diseases of the central nervous system (CNS) or must to reach the CNS to achieve their therapeutic effect. Anti-infectives and drugs used in oncology also contribute substantially to the total approvals. The number of analgetics (predominantly drug products containing paracetamol) approved in Europe (19% of all approvals) seems high when compared to analgetics approved by the FDA (~2%). This can be explained by the fact that, for the approval of generic drug products containing paracetamol, the FDA allows other comparative dissolution testing methods that are not cited in the approval statistics as a BCS-based Biowaiver.[7] Underlining the potential for a BCS-based Biowaiver of essential medicines, the top 3 therapeutic classes of drugs listed on the EML (anti-infectives, oncology, and CNS drugs) are also found among the top 4 therapeutic classes of drug products approved via BCS-based Biowaiver in the United States and Europe.

The high number of oncology drugs approved via BCS-based Biowaiver may seem unusual at first glance because of the critical indication and a high possibility of adverse effects, but as pointed out by Tampal et al.[47] in 2015, oncology drugs are ideal biowaiver candidates, mainly because of the ethical problems of conducting BE studies with cytotoxic agents being administered to healthy volunteers, but also because of other issues such as high variability in BE studies and cost-intensive and time-consuming multicenter studies. An assessment of possible savings in study costs (~60-70 million dollars per year[48]) and other benefits of applying the BCS-based Biowaiver instead of *in vivo* BE studies has been made by Polli,[49] Cook et al.,[48,50] and Arrunátegui et al.[51] The main beneficial points addressed were a more direct comparison of product performance, avoidance of high failure rates of highly variable drugs in BE studies (and thus avoidance of unnecessary testing in humans) as well as better public access to medicines. Certain drawbacks of the procedure were also discussed, namely the lack of global harmonization of the criteria and uncertainty of regulatory acceptance of the submitted data, which could delay drug approval if the regulatory authority demands additional data.[50]

Applicability for Drugs on the Essential Medicines List

To determine the number of essential drugs that are eligible for a BCS-based Biowaiver, the literature was searched for previous BCS classifications and solubility determinations of the 169 orally administered drugs formulated in immediate-release solid dosage forms listed on the 20th EML. The highest dosage strength listed on the 20th EML was divided by the lowest solubility data found in the literature to calculate the dose/solubility ratio and to establish a provisional solubility classification.[22,24,52-61] The search revealed that 94 of 169 drugs (~56%) can be classified as highly soluble (i.e., exhibit a dose/solubility ratio ≤250 mL) and therefore more than half of all orally administered essential drugs with systemic therapeutic effect are possible candidates for a BCS-based Biowaiver generic approval (Fig. 3). A complete list of the drugs and their solubility classification can be found in the supplementary material.

Role of Biowaiver Monographs for the BCS-Based Biowaiver

46 biowaiver monographs have already been issued by the International Pharmaceutical Federation.[52] Those monographs carefully evaluate the possibility of applying the biowaiver procedure to formulations of a specific drug, taking into account the physicochemical and pharmacokinetic characteristics of the drug as well as safety considerations for the patient. Most of the monographs (38 of 46) address drugs that are listed on the 20th EML. Of the 94 highly soluble drugs (excluding vitamins, narrow therapeutic index, or locally acting drugs) on the EML, 26 have already been evaluated in BMs and have received a positive recommendation for a possible approval via BCS-based Biowaiver. Highly soluble drugs listed on the EML that received a negative decision in a BM are quinine and ribavirin, due to dose related toxicity problems (quinine) or high variability as well as narrow therapeutic index (ribavirin).

In addition to the biowaiver monographs, possible approval via BCS-based Biowaiver is mentioned in 34 product-specific BE guidelines of the FDA[7] and in 12 guidelines issued by the EMA.[8] Nine of those product-specific BE guidelines are drugs listed on the EML.

Taking together Biowaiver monographs with a positive decision as well as the former list of possible BCS-based Biowaiver candidates of the WHO,[62] approved drug products in the EU or United States and product-specific BE guidance documents, there is scientific and regulatory consensus for a potential generic approval via BCS-based Biowaiver for 78 APIs, 35 of which are highly soluble APIs listed on the 20th WHO EML. In addition, the FDA recommends specific *in vitro* comparative dissolution methods for demonstrating BE of oral drug products containing hydralazine and vancomycin. Thus, in summary, a complete scientific and regulatory affirmed risk-benefit analysis for a potential waiver of BE studies

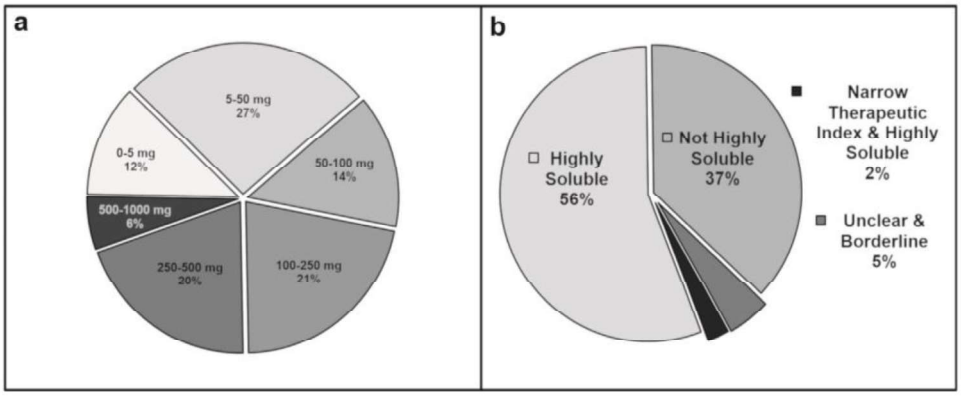

Figure 3. (a) Dosage strength distribution of orally administered drugs on the 20th WHO EML. (b) Provisional solubility classification of 169 orally administered drugs on the 20th EML based on literature and experimental data.

already exists for 37 of the 94 eligible drug candidates on the EML (~39%). The number is likely going to rise even further, with the FDA now allowing BCS class III biowaivers, the WHO planning a revision of their biowaiver list from 2006[53] and more biowaiver monographs on essential drugs being issued by the International Pharmaceutical Federation.

A table with all APIs eligible for a BCS-based Biowaiver or approval via other *in vitro* release testing methods can be found in the supplementary material.

Are BCS-Based Biowaiver Decisions Reliable?

The current, mostly harmonized guidance documents on the BCS-based Biowaiver reflect the scientific consensus that has been achieved in the last decade. Almost all major suggestions for guidance revision proposed by Yu et al.[63] in 2002 and also discussed by Dressman et al.[64] in 2001 were put into practice in the current FDA guidance document: narrowing the solubility pH range to 1.0-6.8, reducing the permeability cutoff to $f_a \geq 85\%$ and allowing BCS class III drugs to be considered for a BCS-based Biowaiver. Other suggestions of Yu et al.,[63] for example, increasing the volume for D/S calculation to 500 mL and allowing bile salts in solubility and dissolution experiments, have not found their way into the BCS-based Biowaiver, but are addressed in the developability classification system[65] (DCS) and its refined version[66] (rDCS). In the period between the proposed changes and the revision of the FDA guidance, the dissolution criteria for BCS class I drugs were affirmed,[67,68] the possibility of including BCS III drugs was positively received in the scientific literature,[69-73] and possible extensions to the dissolution criteria of the EMA guidance document for BCS III drugs were proposed (Table 2).

As pointed out earlier, with the now better harmonized guidance documents, the large number of (essential) drug candidates eligible for the BCS-based Biowaiver, along with scientifically substantiated support and rising approval numbers, the potential of the BCS-based Biowaiver seems greater than ever. Naturally, the question arises whether the biowaiver procedure is discriminative enough, as this is the main requirement for a reliable surrogate for *in vivo* BE studies.

To assess the risk associated with applying the biowaiver procedure, 2 undesirable possible outcomes have to be defined: false-negative (when drug products that demonstrate *in vivo* BE show dissimilar dissolution behavior *in vitro*) and false-positive outcomes (when drug products that are not bioequivalent *in vivo* show similar dissolution behavior *in vitro*). To assess the overall risk of a drug product to fail in a PK-BE study per BCS class, several studies have compiled and analyzed a large number of case examples (ranging from 124 to 918 BE studies).[74-76] All of them found a low risk of BCS I and III drugs failing to demonstrate BE in *in vivo* studies (failure rate ranging from 11% to 16%), implying that a false-positive decision in a biowaiver procedure would be rather unlikely, based on the fact that non-BE rarely occurs for BCS I and III compounds. Nevertheless, examples of false-positive outcomes were found, even in sufficiently powered studies.[74] It needs to be mentioned though, that all studies calculated the occurrence of undesirable outcomes based on dissolution results obtained from single medium quality control methods rather than using the BCS-based Biowaiver multimedia dissolution criteria, thus raising the question whether the same failures would have been obtained with a true BCS-based Biowaiver procedure.

Ramirez et al.[74] found 4 cases where false-positive outcomes occurred with quality control dissolution methods (Table 3). Cólon-Useche et al.[77] discussed 3 of those cases, pointing out that non-BE results for codeine could have resulted from dosing problems in the study, where a liquid, fixed-dose combination with ibuprofen was used (a dosage form to which the BCS-based Biowaiver cannot be applied). A second product discussed was an isoniazid formulation which contained mannitol as an excipient and would thus not comply to the excipient criteria of the current BCS-based Biowaiver guidance documents. Third, the study investigated a false-positive case example involving zolpidem, applying BCS-biowaiver-conform dissolution tests to non-BE zolpidem formulations. As differences in disintegration and dissolution were only found when using low rotational speeds (30 rpm), the authors suggested that paddle speeds higher than 50 rpm should not be used in biowaiver experiments, even if coning occurs. However, a rotational speed of 30 rpm in the paddle apparatus is generally deemed to be unsuitable because the hydrodynamics are very poor and coning (an *in vitro* artifact, i.e., irrelevant *in vivo*) is more pronounced.

In another study, Cristofoletti et al.[76] reported that in 19 of 201 cases where BCS I and III products showed similar dissolution results obtained with single medium quality control methods, the products were deemed non-BE in pharmacokinetic trials, resulting in an overall ~7.5% false-positive rate. The false-negative rate was found to be very low (4% for BCS I drugs, 8% for BCS III drugs) when the "rapid dissolution" criterion of BCS I drugs was applied for BCS III drugs ($\geq$85% dissolution in $\leq$30 min + f_2-test), but rose to 25% for BCS III drugs when the current "very rapid dissolution" criterion

Table 2
Potential Extensions of Guidance Criteria as Suggested in the Literature

Drug and BCS Class	Summary of Main Findings	Reference
Potential extensions of current guidance criteria		
Metoprolol tartrate—BCS I	QC Dissolution specifications for metoprolol tartrate may be widened to "complete release in 60-90 min" and still assure BE.	Polli et al.[111] (1997)
BCS I and III drugs	PK simulations support that current regulatory criteria are conservative, BE can be achieved for some drugs even when dissolution time is 30-60 min.	Tsume et al.[112] (2010)
BCS I and III drugs	Proposal of individual specification adjustments based on t_{max} and $t_{1/2}$.	Kortejärvi et al.[91] (2014)
Metformin—BCS III	Dissolution time specification of <30 min is appropriate for this BCS III drug, differences *in vitro* do not reflect changes *in vivo*.	Cheng et al.[113] (2004); Homsek et al.[114] (2010)
Metformin—BCS III	*In silico* modeling supports that release times up to 2 h do not significantly impact PK parameters.	Crison et al.[115] (2012)
Metformin—BCS III	4 Products were tested in different set-ups: USP II apparatus at 50 rpm was most sensitive, differences in release time up to 30 min will not affect PK parameters significantly.	Ardelean et al.[116] (2018)
Cimetidine—BCS III	*In vitro* and *in vivo* studies support that >85% dissolution in <30 min is sufficient to guarantee BE of IR formulations of cimetidine.	Jantratid et al.[117] (2006)
Carbamazepine—BCS II	IVIVC is possible using dissolution media with 1% SLS, coupled with PK modeling, biowaiver might be possible, but the drug is considered to have an NTI.	Kovačević et al.[118] (2009)
Etoricoxib—BCS II (weak base)	API dissolves in acidic media and stays supersaturated—possible BCS II biowaiver candidate supported by PK modeling.	Okumu et al.[119] (2009)
BCS II drugs (especially Ibuprofen)	Proposal of a discriminative biorelevant dissolution method for ibuprofen using biorelevant media, coupling of *in vitro* results with *in silico* PBPK and PD modeling to demonstrate pharmacokinetic and therapeutic equivalence.	Cristofoletti et al.[92,108,120] (2014, 2016, 2017)
BCS II weak acids	Theoretical model for predicting complete absorption of weakly acidic drugs based on physicochemical and PK parameters.	Rinaki et al.[121] (2004)
18 BCS II weak acids	15/18 weakly acidic BCS II drugs could be classified as BCS I when only considering solubility at pH 7.4; intermediate solubility classification is proposed for such drugs to allow biowaivers.	Yazdanian et al.[122] (2004)
BCS II weak acids and bases	PK modeling is useful to identify BCS II biowaiver candidates; some BCS II candidates require discriminative dissolution methods and IVIVC.	Tubic-Grozdanis et al.[123] (2008)
BCS II drugs	Subclasses for BCS II and IV drugs are proposed to identify possible biowaiver candidates among BCS II drugs, sufficiently discriminative dissolution methods are needed to support biowaiver decisions.	Tsume et al.[124] (2014)
BCS III drugs	PK simulations support widening the dissolution time criteria for BCS III drugs to 30 min, proposal of permeability boundaries for an intermediate permeability class (30% <F_a< 85%).	Sun et al.[125] (2017)

IR, immediate release; IVIVC, *in vitro in vivo* correlation; NTI, narrow therapeutic index; PD, pharmacodynamic; QC, quality control.

was applied (≥85% dissolution in ≤15 min), simultaneously reducing the false-positive rate from 7% to 4%.

Again, it must be pointed out that in each of these studies, all dissolution test results that were compared with the PK-BE study results were obtained from quality control methods. As the BCS-based Biowaiver-conform dissolution tests are expected to be more discriminative due to testing in multiple media, the false-negative rates reported so far are likely underestimates, whereas the number of false-positive case examples is likely overestimated (also considering that, as reported by Cólon-Useche,[77] some of the reported false-positives could simply not have been approved via the BCS-based Biowaiver).

Table 3
Case Examples of False-Positive and False-Negative Outcomes of *In Vitro* Dissolution Tests When Compared to BE Study Results

Drug and BCS Class	Summary of Main Findings	Reference
False-positive examples (non-BE products were found to be equivalent *in vitro*)		
Zolpidem—BCS I	Non-BE products were found to be *in vitro* equivalent at paddle speeds >50 rpm.	Colón-Useche et al.[77] (2015)
Dexketoprofen—BCS I	Non-BE products were tested, *in vitro* methods were not discriminative enough at higher paddle speeds (>50 rpm), time until complete dissolution should be <30 min.	Garcia-Arieta et al.[78] (2015)
Pravastatin, Zolpidem, Codeine—BCS I; Isoniazid—BCS III	False-positive outcomes were found for those APIs in sufficiently powered BE trials, *in vitro* methods were QC methods.	Ramirez et al.[74] (2010)
Ibuprofen—BCS II	Comparison of ibuprofen test and reference formulations in human BE trials and via dissolution testing: lack of discriminatory power of the *in vitro* tests, no rank-order correlation could be made. Reference products contained SLS!	Alvarez et al.[79] (2011)
False-negative examples (BE products do not meet criteria or were found to be inequivalent *in vitro*)		
Amoxicillin—BCS I	Generic products showed differences *in vitro* and did not meet the specifications.	Zuo et al.[80] (2014)
Droxidopa, Famotidine, Fexofenadine, Hydrochlorothiazide—BCS III	Oral disintegrating tablets were compared to IR formulations. For the drugs in column 1, dissimilar dissolution but bioequivalence *in vivo* was observed.	Ono et al.[81] (2014)
Metronidazole, Amoxicillin—BCS I	Comparison of generic drug products from different countries, several examples of differences in dissolution time and failure to meet regulatory dissolution criteria were observed.	Reddy et al.[41] (2014) Reddy et al.[42] (2016)
Acyclovir, ciprofloxacin hydrochloride—BCS III		

IR, immediate release; QC, quality control.

BCS-Biowaiver-Conform Dissolution Tests Performed at the Goethe University and Observed Failure Rates

To provide a more reliable estimate of the false-negative rate of the BCS-based Biowaiver, dissolution tests with essential drugs that were performed over the last decade at the Goethe University, Frankfurt am Main, Germany, were summarized.[54,82-84] Based on literature data, the drugs were assigned to the appropriate BCS class using a conservative approach (i.e., in case of borderline behavior, a "worst-case" classification was made) and the performance of drug products was evaluated based on specifications in the regulatory guidance documents, without comparing the products to a reference product. An overview of the outcome of this retrospective analysis is shown in Figure 4. Nine of the 16 APIs were classified as highly soluble drugs, and a total of 45 dissolution tests with drug products containing BCS class I or III APIs were found. Thirteen drug products failed to comply with the guidance specifications, and 2 showed borderline behavior, although in every case, dissolution of the pure API was able to meet the criteria. The APIs with the highest failure rates were ethambutol dihydrochloride (BCS III, 5/7) and doxycycline hyclate (BCS I, 3/9). Regarding the dissolution tests with BCS class II/IV APIs, all drug products failed in the BCS-Biowaiver-conform dissolution tests. In addition to the BCS II/IV drug products that were tested over the whole pH range of 1.2-6.8, several drug products containing ibuprofen were only tested in media at pH 6.8 and some showed ≥85% dissolution in 15 min at that pH. However, for this weakly acidic drug it is expected that the drug products would fail to comply with the guidance criteria at more acidic pH values, as the D/S is >4 L in media below pH 4.5.[85]

Considering a "best case" approach—a drug product that complies with the regulatory dissolution criteria is deemed *in vitro* equivalent with a fictitious comparator product that also passes the criteria—the 95% Clopper-Pearson interval for the combined false-negative rate is ~17%-44% (Fig. 4), with a point estimate of 29%. This rate is comparable to the rates reported in the studies mentioned previously (≤25%, even with the strictest criteria) and is expected to be even higher under conditions where the generic products are actually compared to a reference product, thus allowing products to individually meet the regulatory conditions, but still fail the test of similarity with the comparator. In line with the finding that the BCS-based Biowaiver approach demonstrates a high false-negative rate, there are numerous reports in the literature that the current guidance specifications are overly strict in regard to BCS class III compounds, and that wider specifications should be considered in future revisions (Table 2).

Unfortunately, because of the practical difficulty of identifying non-BE BCS I and III drug products, no statistically sound estimate of the prevalence of false-positive results could be made. Just a few examples can be found in the literature for the occurrence of false-positive outcomes under biowaiver conditions (Table 3) or the absence thereof.[86]

PBPK and PK/PD Modeling to Establish Clinically Relevant Dissolution Criteria

As discussed in a previous section, there is still room for improving the discriminatory power of the BCS-based Biowaiver procedure for class I and III drugs, as there is a high incidence of false-negative outcomes along with sporadic reports of false-positive outcomes reported in the literature. The 'one size fits all' approach currently applied by the regulatory guidance document seems appropriate in most cases, but may be less suitable for certain categories of API. The need for individual modifications to regulatory criteria for demonstrating BE has also been reviewed by Cristofoletti et al.[87] Focusing on the current approaches to demonstrate BE, the authors state that the currently applied "average bioequivalence" criterion may have to be challenged in favor of drug-specific regulations, taking into account individual parameters significant to therapeutic equivalence. This statement is not only true for the traditional BE, but also for the BCS-based Biowaiver approach. The question arises, which specific properties of an API may lead to the regulatory guidance documents being unsuitable.

Most drug products that fail to demonstrate BE *in vivo* do so because they do not meet the requirements for the confidence interval of C_{max}.[74,76] Other reviews concerned with the analysis of risk factors in BE studies further substantiate the impact of C_{max} on the outcome of the studies, stating that in many cases, C_{max} is more variable than the area under the plasma drug concentration-time curve, and high variability is always a major risk factor for bioinequivalence as a result.[88,89] Thus, to answer the question of API properties that may lead to false decisions in a BCS-based

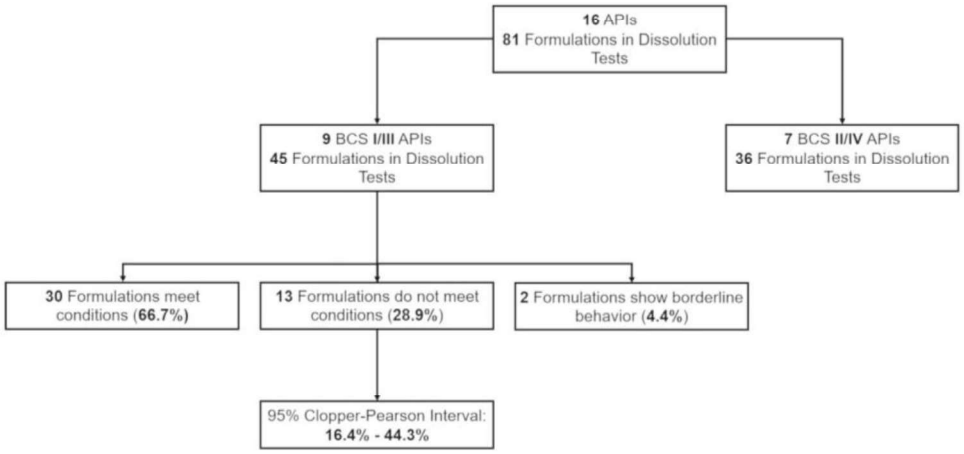

Figure 4. Outcome of dissolution tests under BCS-biowaiver conditions performed with essential medicines at the Goethe University, Frankfurt am Main, Germany (full list can be found in the supplementary material).

Biowaiver, properties that influence C_{max} have to be considered as critical. Physiological influences on the C_{max} of a drug product are, for example, gastric emptying time, prandial state, body position (may affect gastric emptying time), and volume of intestinal fluids. Factors inherent to the API are the pharmacokinetic half-life, solubility in the GI tract, and the effective permeability in the small intestine, whereas formulation-related parameters are the wettability, particle size, disintegration time, and dissolution rate. The key principle of the BCS-based Biowaiver is to compare the formulation performance of 2 drug products. High variability in gastric emptying time, high effective permeability of a drug, and short pharmacokinetic half-life may combine to cause false-negative BE results *in vivo*. In theory, drugs for which the BCS-based Biowaiver may not be discriminative enough are therefore highly permeable, highly soluble APIs with short pharmacokinetic half-life.[90] Those theoretical assumptions are substantiated when looking at the case examples of false-positive biowaiver outcomes (Table 3) and their pharmacokinetic parameters (Table 4). All drugs are highly soluble at the pH of the small intestine, most of them (except for isoniazid) are high permeability drugs and all of them show a short pharmacokinetic half-life and an early t_{max}. For these and other APIs with similar properties, adjustment of regulatory dissolution specifications may be necessary. For certain BCS class I drugs, there are suggestions in the literature of either just allowing the very rapid dissolution criterion (≥85% release in 15 min),[91] or to limit the media volume and rotational speed in the dissolution experiment (e.g., no more than 500 mL and 50 rpm).[77,78] Narrowing or widening regulatory dissolution criteria should preferably be supported by modeling and simulation to validate specifications based on the individual parameters that affect BA for the specific drug the most.

Regarding the occurrence of false-negative biowaiver outcomes, the guidance specifications may be overly strict for drug products containing APIs with a long pharmacokinetic half-life and permeability-limited absorption, as is the case of many BCS class III drugs. In fact, many authors have suggested wider dissolution specifications for BCS class III drugs (Table 2) or even consider BCS class III drugs as the better candidates for a BCS-based Biowaiver compared to BCS class I drugs.[69]

To investigate possible extensions to the current biowaiver criteria and to establish individual dissolution specifications, *in silico* modeling approaches such as PBPK or pharmacodynamic modeling has been utilized by many authors.[92-95] Using *in silico* modeling approaches, a possible impact of differences observed in biorelevant dissolution tests on pharmacokinetic or pharmacodynamic outcome parameters can be evaluated, and recommendations for individual adjustments for regulatory dissolution criteria can be made. Although there is still some hesitation to the use of *in silico* tools in the interaction between regulatory authorities and pharmaceutical companies, it is expected that the influence of those tools and their acceptance will grow larger in the future.[96-98] A recent study conducted by Pepin et al.,[99] although not associated with the BCS-based Biowaiver, demonstrated that "safe-space" dissolution criteria for ensuring BE that were generated from experimental data and *in silico* modeling approaches are indeed feasible and have been accepted by regulatory authorities. The study by Pepin et al. demonstrates that risk assessment of differences in dosage form performance and the subsequent decision of whether to perform a BE study is possible based on validated PBPK models, and may also be a viable approach for granting BCS-based Biowaivers in the future.

Table 4
Selected Pharmacokinetic Parameters[126] of Drugs With Documented Occurrence of False-Positive BCS-Based Biowaiver Outcomes

Drug Name	Time of Maximum Plasma Concentration t_{max} [h]	Elimination Half-Life $t_{1/2}$ [h]
Zolpidem	1.0-2.6	1.9 ± 0.2
Pravastatin[a]	1-1.4	0.8 ± 0.2
Codeine[a]	1.0 ± 0.5	2.9 ± 0.7
Isoniazid[a]	1.1 ± 0.5	1.1 ± 0.1 (3.1 ± 1.1 for SA)
Dexketoprofen	1-2	2
Ibuprofen	1.6 ± 0.3	2 ± 0.5

SA, slow acetylators.
[a] False positive result obtained with QC testing, not with BCS based Biowaiver testing.

Inclusion of BCS Class II Drugs via Biorelevant Dissolution Testing

Although generally well accepted for BCS class I and III drugs, the inclusion of BCS class II drugs in the BCS-based Biowaiver procedure is controversial. Based on the fact that, for certain BCS class II weakly acidic drugs that are highly soluble in the physiological environment of the small intestine, the only limiting factor for the compounds' absorption would be the gastric emptying time, the WHO included this group of compounds in their first Biowaiver guidance.[4] This approach was not continued in their revised guidance document, partly because of concerns raised by García-Arieta et al.[79,100,101] The dissolution methodology stated in the guidance documents was found not to be sufficiently discriminative to reliably distinguish between products that were deemed not to be bioequivalent in human BE studies. The most discussed case is ibuprofen, for which 2 studies concluded on the one hand that the regulatory criteria were either overly discriminative[102] and on the other hand not discriminative enough.[79] Variations to the methodological dissolution setup and coupling the *in vitro* results with *in silico* modeling may be ways forward to overcome these issues (Table 2).

As opposed to BCS class I and III compounds, for which the regulatory dissolution methods are generally suitable and only the dissolution time specification may need to be revised for certain APIs to guarantee the discriminative power, the regulatory methodological setup seems unsuitable for class II drugs. Testing the drug products of BCS class II compounds in various media separately, as required in the guidance documents, is not likely to reflect the *in vivo* behavior because a critical step for dissolution is the transition from the gastric compartment into the small intestinal compartment for many BCS class II weak acids and bases. To reliably assess the performance of a BCS class II drug product, it is therefore necessary to apply methods that take into account the transition between compartments, such as 2-stage tests,[103] the transfer model,[104] or other methods, as reviewed by Kostewicz et al.[105] Furthermore, the use of compendial buffers such as simulated intestinal fluid or Ph. Eur. buffers may be unsuitable for many class II APIs because of their higher buffer capacity compared to physiological fluids. This may influence the surface pH and thus the dissolution rate. Another important aspect is the lack of bile salt components, which can have an important influence on the solubility. Therefore, also following recommendations of Markopoulos et al.,[106] it seems reasonable to modify the dissolution media for BCS II compounds, taking into account not only pH and buffer capacity, but also bile components and osmolality. As an example, Cristofoletti et al.[107] demonstrated that when using biorelevant media that were adjusted for buffer capacity, a more discriminative dissolution method for ibuprofen could be established.[108] Although the reduced buffer capacity has some drawbacks, such as the need to adjust the bulk pH of the media throughout the experiment, it serves as a valuable case example for possible further, scientifically based extensions of the BCS-based Biowaiver. More research in this

area with different model drugs would be of great importance to demonstrate that those findings can also be transferred to other weakly acidic BCS class II drugs.

In summary, in the light of the last WHO world medicines situation report[109] from 2011, ongoing research aiming to expand the application of the BCS-based Biowaiver and to ensure its discriminatory power is contributing to achievement of the central aim of the WHO Essential Medicines and Health Products Department, that is, "increasing the access in all countries to essential medicines and other health products."[110] As of 2011, the WHO stated that the share of essential medicines of all drug products used worldwide was 25%-35%, global medicine availability was below 60% (and as low as 32% in the eastern Mediterranean region) and acute infectious diseases as opposed to chronic diseases still remain the major threat in low- and middle-income areas of the African region.[109] Promoting the concept of an internationally harmonized BCS-based Biowaiver for essential drugs along with a global comparator product list would not only contribute greatly to the availability of affordable generic drug products of essential medicines while ensuring equal quality in all regions but also reduce the regulatory burden and the expenditures and ethical issues associated with human BE trials.

Conclusion

Over the last decade, the relevance of the BCS-based Biowaiver to the approval of generic drug products has increased greatly. The recent ICH draft guidance contributes to the ongoing and much needed international harmonization of the various guidance documents on BE testing, but still has potential for substantial improvement. Closely linked to harmonization of the guidance documents, internationally acknowledged comparator products for the BCS-based Biowaiver are desirable to globally align the performance and quality of generic drug products. The scientific and regulatory consensus of allowing BCS III drugs for the procedure increases the number of potential drug candidates for a biowaiver markedly, and yet has been proven to be sufficient in most cases and even overly discriminative in some. However, problems occur occasionally with few a APIs (false-positive and false-negative examples are documented) and specific excipients. Therefore, further studies on the biowaiver procedure for individual APIs and drug products are needed to investigate the possibility of modifying the regulatory specifications for comparative dissolution testing. Related to validating the method for BCS class I and III drugs, more research is necessary to find scientific consensus on the controversial topic of excipient effects. Application of the procedure to BCS class II drugs, which would be desirable to further increase the number of drugs that could potentially be approved via BCS-based Biowaiver remains controversial and should also be the topic of future research.

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A.1.3.4 Publication 4

Differences in drug quality between South Africa and Germany

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Differences in drug quality between South Africa and Germany

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Abstract

Objectives To examine differences in drug product quality between products marketed in developed countries and in developing countries.

Methods The quality of drug products marketed in both Germany and South Africa by the same pharmaceutical company was compared. A fixed-dose combination tablet containing amoxicillin/clavulanic acid, and mometasone furoate nasal spray were selected to represent generic medicines requiring prescriptions, while skin lightening products (legally obtained and/or confiscated) were selected to represent pharmaceutical products that are available without a prescription. Pharmacopoeial tests included assay, content uniformity, and where applicable, dissolution in addition to a visual examination of the packaging.

Key findings Some differences between the product marketed in Germany and in South Africa were detected for the amoxicillin tablet formulations, although all samples still complied with regulatory requirements. The mometasone nasal spray product marketed in South Africa delivered a higher dose than was declared on the label. The composition of the skin lightening products conformed qualitatively with labelling, but in some South African samples alarmingly high amounts of hydroquinone were found.

Conclusions Important differences in quality were detected between some German and South African products. To preclude drug products of poor or doubtful quality from entering the market in South Africa, countermeasures are needed.

Introduction

As is the case for other types of products, the pharmaceutical market has changed rapidly in recent years due to globalization. Up till the time when a patient receives a medicine, its components are likely to have travelled long distances, with several sites or even companies involved. As a result, national regulatory authorities not only have to monitor pharmaceutical manufacturers within their borders, but also all imports and associated manufacturers from abroad. Additionally, the digital revolution has facilitated growth in the number of patients who buy their medicines online, some of which may come from overseas. Consequently, custom agencies have had to intensify their border controls.[1–3] Several experts, lay articles and scientific publications have suggested that the discrepancies in the monitoring of drug product quality and legislative framework that exist between countries can have an impact on the quality of pharmaceutical supply.[4,5]

Standards for drug quality are set by internationally accepted pharmacopeias such as the United States Pharmacopiea (USP) and the European Pharmacopeia (EP), as well as by national laws and regulations. At the national level, the manufacture, import and export of medicines are regulated to assure adequate provision of medicines to the public. These regulations represent the legal basis for control of the distribution chain and for verification of compliance with quality regulations put in place by the governmental authorities. The protection of public health against poor quality drugs is a major goal of every national authority.

'Poor quality drugs' is a collective term for legal (and illegal) medicinal products which do not comply with regulations.[6–8] While legal medicines can be further divided into

substandard or degraded medicines, illegal medicines comprise non-approved, counterfeit and falsified products. A characteristic aspect of illegal medicines is the lack of official marketing approval in the country where they are offered for sale. Many illegal medicines exhibit inappropriate chemical, microbiological and/or physical properties, such as containing the wrong API (active pharmaceutical ingredient), containing the wrong dose of the API or containing chemical or microbiological contaminants.[9 13] Legal poor-quality drugs, on the other hand, are approved medicines which are characterized by inadequate quality due to improper production, transport or storage conditions. Although most countries have defined and introduced legislation against poor quality drugs, these regulations sometimes differ substantially from one country to another.[14 16] Consequently, it is no surprise that international organizations like the WHO are still discussing, changing and clarifying definitions.[17,18] In this publication, we use the definitions shown in Table 1.

To date, there have been very few studies comparing the quality of drug products produced for different countries by the same pharmaceutical manufacturer. Indeed, to the best of our knowledge, the current study is the first one which analyses medicines and cosmetics from Germany (representing developed countries) and South Africa (considered to be a developing country) to evaluate whether these countries are supplied with the same quality of drugs.[19] In order to consider the diversity of the pharmaceutical supply chain, two types of products were evaluated. First, approved generic medicines produced by prominent manufacturers in India and sold in both South Africa and Germany were sampled. These products were Amoxiclav from Aurobindo Pharma Limited and mometasone furoate nasal spray from Cipla Limited. Amoxiclav is a fixed-dose combination (FDC) antibiotic product consisting of amoxicillin and clavulanic acid. Amoxicillin is a broad-spectrum antibiotic agent used against several types of bacterial infection, while clavulanic acid is added as a β-lactamase inhibitor to reduce the probability of resistance.[20] Mometasone furoate applied as a nasal spray is used for the treatment of allergic rhinitis. The corticosteroid exhibits anti-inflammatory effects and is therefore mainly used for the treatment of allergies.[20]

Table 1 Definitions used for poor quality drugs in this publication[7,9,17,18,48,49]

Poor quality products	A collective term for all drugs not fulfilling the pharmacopoeial requirements or violating the national laws. It is an umbrella term for counterfeit, fake, degraded and substandard drugs.
Illegal products	Drugs which do not have official approval or registration. These products are therefore distributed outside the national legal framework for medicines.
Counterfeit drugs	Deliberately and fraudulently mislabelled drugs with respect to identity and/or source. A synonym for fake drugs.
Substandard drugs	These products do not fulfil the requirements of the pharmacopoeia, but are produced by the registered pharmaceutical manufacturer. The company is responsible for the quality defects.
Degraded drugs	Products which were produced by the registered pharmaceutical manufacturer, but due to inappropriate storage after batch release, the drugs no longer fulfil the pharmacopoeial requirements.

Additionally, illegal medicines that had been confiscated by the South African border authorities were compared to similar products marketed in Germany. The South African skin lightening products contained either highly potent topical corticosteroids such as clobetasol propionate and betamethasone, or hydroquinone and/or kojic acid, both of which inhibit melanogenesis either via inhibition of the key enzyme tyrosinase (hydroquinone and kojic acid) or by lowering the levels of melanocyte-stimulating hormone via a negative hormonal feedback (corticosteroids).[21 23] There is a high risk of side effects associated with the use of such skin lightening agents, especially when used over a long period. These side effects include skin atrophy and endocrinological disruption (the typical complications of topical steroids) irritant contact dermatitis (kojic acid, hydroquinone) and post-inflammatory dyspigmentation (hydroquinone), which may be manifested as exogenous ochronosis.[21 23]

Methods

Sampling

To investigate differences between the German and South African pharmaceutical supply chain, prescription medicines produced by two globally acting companies based in India were purchased through reputable wholesalers. The illegally distributed skin lightning products examined in this study were confiscated at the South African border control and compared with products that had the same outer appearance and were legally obtained on the German market.

Prescription medicine

As in a previous study it had been determined that poor storage conditions in some pharmacies in South Africa may lead to poor drug quality,[24] it was decided to purchase the prescription medicines from wholesalers in South Africa (EDNA Medical Distributors) and Germany (PHOENIX Pharmahandel GmbH & Co KG). This procurement strategy also avoided any selection bias that might have arisen if the company had been directly approached to provide samples.

Choice of manufacturers was based on the global presence of the pharmaceutical company and whether it

distributed the given products at the same dosage strength in both South Africa and Germany. The first two products identified, which were able to fulfil these criteria, were selected.

1) Tablets containing amoxicillin and clavulanic acid produced by Aurobindo (Germany: Amoxi-Clavulan Aurobindo 500 mg/125 mg – PZN: 09425333 – RegNr: 78579.00.00; South Africa: AURO AMOXICLAV 625 mg – RegNr: 41/20.1.2/0536).
2) Mometasone furoate nasal spray produced by Cipla (Germany: Mometasonfuroat Cipla, 50 µg/metered spray, 18 g – PZN: 10780163 – RegNr: 90078.00.00; South Africa: Nexomist 50 µg/metered spray, 18 g – RegNo: A38/21.5.1/0341).

Skin lightening products

A different approach was used for the skin lightening products. The Department of Health in South Africa provided two bags of skin lightening products which had been recently confiscated. These products were seized at airports by customs officials during attempted import into South Africa and subsequently provided to our research group through official channels. At this point, it should be mentioned that confiscation of such products is not a rarity in South Africa, and it is suspected that large numbers of such products enter the country, undetected by South African customs.[25] Skin lightening products are in widespread use in South Africa, and are frequently sold in 'spaza' shops (a type of informal convenience store) and public markets. The German counterpart products were located and purchased in 'afro' shops (shops specializing in products for African customers) close to the main railway station in Frankfurt am Main. The appearance of the packaging and the purported name of the manufacturer was close to identical, as shown in Figure 1 and Table 2. Product sources were purported to be from both developed and developing countries.

Chemical analysis and quality control tests

All products were chemically analysed with HPLC-UV/Vis (VWR Hitachi, Darmstadt, Germany). For all APIs, HPLC methods were developed or adopted and validated according to ICH-guidelines. Table 3 provides an overview of the method parameters as well as validation data for the prescription medicines, while Tables 4 and 5 depict the same information for the skin lightening products.

Prescription medicines

Amoxiclav. The content of a South African ($n = 10$, FN5016003-A) and a German batch ($n = 10$, EA5016021-G) of Amoxiclav tablets was determined. Each tablet was dissolved in 1 l of purified water in a volumetric flask, which was then placed on a magnetic stirrer (500 rpm) at room temperature for 30 min, followed by ultrasonication for 10 min. After filtration (0.45 µm PTFE membrane filters; VWR International, Darmstadt, Germany), the samples were analysed via HPLC. The acceptance value was calculated according to the pharmacopoeial chapter 'uniformity of dosage units' (USP <905>)[26]:

$$AV = |M - \bar{X}| + ks$$

where AV: acceptance value; M: reference value; X: mean; k: acceptance constant; s: relative standard deviation.

Additionally, dissolution testing was carried out according to USP 39.[26] Three different batches of the German product (EA5016021-G, EM5016023-C, EM5017004-B) were tested to avoid bias due to incidental outliers. The South African wholesaler was only able to provide one batch (FN5016003-A). To obtain a better overview of product performance, the results were compared to a study performed in the same year with samples from the same manufacturer but purchased in community pharmacies in South Africa.[24]

Six individual tablets were introduced into USP II apparatus (ERWEKA DT 720 dissolution tester, ERWEKA GmbH, Heusenstamm, Germany) and allowed to dissolve over 30 min. Prior to analysis, the equipment had been qualified mechanically and performance verification had been performed in accordance with USP.[27] Following the product specification, the tests were carried out with 900 ml water at 37 ± 0.5°C and a paddle rotation speed of 75 rpm. The dissolution medium was degassed via vacuum filtration for 30 min using mixed cellulose ester 0.45 µm membrane filters (ME 25 by GE Healthcare) before transfer into the vessels. If the batch did not fulfil the compendial criteria for stage 1, that is 90% release for amoxicillin and 85% for clavulanic acid, stage 2 was performed ($Q_{Amoxicillin}$: 85% and $Q_{Clavulanic\ acid}$: 80%).[26]

To further compare the release of amoxicillin and clavulanic acid from the German and South African drug products, their dissolution profiles in SIF_{sp} pH 6.80 ± 0.05 were also investigated. The same dissolution apparatus and parameters as used for the USP quality test were used. Tablets from each batch were investigated ($n = 5$ per batch). The pH 6.8 dissolution test was performed over 1 h. At sampling times (5, 10, 15, 20, 30, 45 and 60 min), 5 ml of media was withdrawn from the vessel and filtered through 0.45-µm PTFE membrane filters. The first 3.5 ml of the filtrate was returned to the dissolution vessel, and the remaining 1.5 ml was transferred into 2 ml microreagent tubes. Immediately after sampling, 0.85 ml of the withdrawn, filtered sample was diluted with 0.15 ml of 0.1 N HCl, to adjust the pH to 6.0 for maximum API stability

Figure 1 Skin lightening products from Germany and South Africa with similar packaging appearance.

during analysis, as previously established in a degradation study at room temperature (unpublished data). No volume compensation for the withdrawn media was carried out, but the amount of API per 1.5 ml filtrate was accounted for in the calculations for subsequent samples.

Mometasone furoate nasal spray. The test of content uniformity was performed for the mometasone nasal spray. Using the method prescribed in the European Pharmacopeia,[28] each sample was shaken for 5 s before triggering one metered dose, which was discarded. This procedure was repeated every 5s for a total of five repetitions. Then, one metered dose was collected in a 10ml volumetric flask. After filling the flask with mobile phase to the nominal volume, the sample was vortexed and sonicated two times for 5 min. After filtration, the mometasone content was measured via HPLC. For the first stage of content uniformity, 10 samples were analysed. If the acceptance value was greater than the limit (L1) of 15%, 20 additional spray doses were tested in accordance with stage 2 of the test.

Skin lightening products

The skin lightening products confiscated in South Africa were inspected visually (including appraisal of the primary packaging, labelling, homogeneity and appearance of the contents), and the main skin lightening ingredients according to the labels (hydroquinone, kojic acid and glucocorticoids) were analysed quantitatively. The sample preparation and content analysis of hydroquinone and kojic acid were adopted from Wang *et al.*[29], while the procedure for the glucocorticoids was adopted from Gaudiano *et al.*[30] (See Tables 4 and 5 for details). The content of skin lightening substances was calculated from the peak area of the respective substance in the chromatogram using the linear calibration curve and divided by the total amount of skin lightening product weighed into the centrifuge tubes to arrive at the percentage in the product. Three individual samples were prepared for each product ($n = 3$).

Data analysis and statistical evaluation

The quality of prescription medicines was evaluated using the pharmacopoeial specifications stated in the USP and EP. Statistical data analysis and graphical representation was performed using SPSS V.24 (IBM Analytics, Armonk, New York, USA) and SigmaPlot 11.0 (Systat Software GmbH, Erkrath, Germany). The measured content of hydroquinone in the examined semisolid dosage forms was compared with the declared amount on the label using Student's *t*-test with Bonferroni's correction.

Results

Amoxiclav

Content uniformity

Both the German and South African product samples passed the pharmacopoeial test for content uniformity,

Table 2 Skin lightening products analysed, along with information taken from the labels

	Product name	Manufacturer according to label	Lot	Amount of hydroquinone	Amount of kojic acid	Amount of corticosteroids
Confiscated in South Africa	Carotone brightening oil	Nouvelle Parfumerie Gandour, Côte d'Ivoire	Unreadable; No NAFDAC Reg. No.	Declared on label (2.0%)	Declared on label	N/A
	Carotone black spot corrector	Nouvelle Parfumerie Gandour, Côte d'Ivoire	D208689"S"; No NAFDAC Reg. No.	Declared on label (2.0%)	Declared on label	N/A
	So White! Skin perfector crème	Labo. Derma, France	156A9	Declared on label (as hydroxyphenol)	N/A	N/A
	So White! Lait skin perfector	Labo. DERMA, France	56317	Declared on label (as hydroxyphenol)	N/A	N/A
	Clear essence medicated fading cream	Bluefield Associates, Inc., Canada	160415-1; NAFDAC Reg. No.: 02-3456	Declared on label (2.0%)	N/A	N/A
	Lemonvate cream	Esapharma srl, Italy	1592038/1592039	N/A	N/A	Declared on label (0.05% clobetasol propionate)
	Movate cream	Esapharma srl, Italy	15100360/1510013	N/A	N/A	Declared on label (0.05% clobetasol propionate)
	Funbact-A triple action cream	Bliss GVS Pharma Ltd, India	FC1649; NAFDAC Reg. No.: 04-6969	N/A	N/A	Declared on label (0.05% betamethasone dipropionate)
	Epiderm lotion	Shalina Laboratories PVT Ltd, India	J5025; NAFDAC Reg. No.: A4-6173	N/A	N/A	Declared on label (0.05% betamethasone dipropionate)
Products acquired in Germany	Carotone brightening body lotion	Mama Africa Cosmetics; Distributor: Alimenti dal Mondo, Italy	13354	N/A	Declared on label (as dipalmitate ester)	N/A
	So White! Skin perfector gel	Labo. DERMA, France	15929	N/A	Declared on label	N/A
	Medicated cleansing bar plus exfoliants	Bluefield Associates, Inc., Canada	150526	N/A	N/A	N/A
	Betavate gel	Cosmo-Black GmbH, Switzerland	514029	N/A	N/A	N/A

N/A no information available on the label.

with an acceptance value lower than 15%. The results were slightly different, but within the expected analytical range. A mean amoxicillin content of 100.8% and a standard deviation of 2.3% resulted in an acceptance value of 5.5% for the German product. The results for clavulanic acid are similar (X: 99.3%; SD: 1.9%; AV: 4.6%). The South African product is characterized by marginally higher acceptance values (amoxicillin – X: 99.4%; SD: 3.9%; AV: 9.4%; clavulanic acid – X: 101.4%; SD: 4.1%; AV: 10.0%).

Dissolution test

Although both products passed the pharmacopoeial dissolution test, clear differences were observed regarding amoxicillin (Table 6). While the German batches passed stage one ($n = 6$, all 6 units to dissolve at least Q + 5 = 90%), stage two dissolution testing ($n = 12$, mean value of Q = 85% dissolved or greater) had to be performed in order for the South African product to pass.

As described in the Methods section, only one batch was accessible through the South African wholesaler. To compensate for this shortage of data, Figure 2 illustrates the mean dissolution results of stage two testing for eight samples of Auro Amoxiclav 625 that had been purchased in community pharmacies in South Africa [24]. The eight samples comprised four different batches (CD5014022-A, FN5016002-A, FN5016003-A, FN5016004-A) of Auro Amoxiclav 625. Comparison of the results in Table 6 and

Table 3 Parameters of HPLC method and validation for amoxiclav and mometasone

Method	Amoxicillin and clavulanic acid		Mometasone furoate
Column	LiChrospher 100 RP-18 (5 μm) LiChroCART 125-4 by Merck KGaA		Purospher STAR RP-18 endcapped (5 μm) LiChroCART 250-4.6 by Merck KGaA
Mobile phase	1 part acetonitrile and 24 parts H_2O 25 mmol KH_2PO_4 pH: 2.5 (adjusted with H_3PO_4)		1 part acetonitrile and 1 part H_2O
Flow rate	1 ml/min		1.25 ml/min
Wavelength	220 nm		249 nm
Sample preparation	Filtrating with a 0.45 μm filter		
Validation	**Clavulanic acid**	**Amoxicillin**	**Mometasone furoate**
Reference standard	Lithium clavulanate CRS Eur. Pharmacopeia L0720000 Batch: 6.0	Amoxicillin trihydrate Sigma Aldrich PHR1127-1G Batch: LRAA8983	Mometasone furoate Sigma-Aldrich PHR1619-1G Batch: LRAA5289
Retention times (min)	3.2	8.4	21.3
Peak symmetry	1.1	1.1	1.1
Resolution	Clavulanic acid compared with amoxicillin: 14 Amoxicillin compared with cefadroxil: 6.7		Mometasone furoate compared with beclamethasone dipropionate: 8.4
Calibration curve	$y = 65\,259x + 200$ (μg/ml)	$y = 56\,581x + 13\,419$ (μg/ml)	$y = 89\,779x - 9993$ (μg/ml)
Calibration curve range	37.5 150 μg/ml	150 600 μg/ml	1 9 μg/ml
Correlation coefficient	1.000	0.9999	1.000
LOQ	<0.75 μg/ml	<3.0 μg/ml	<1.0 μg/ml
Recovery	101.4%	100.0%	97.6%
Repeatability	RSD: 0.3%	RSD: 0.4%	RSD: 0.2%
Reproducibility	RSD: 1.4%	RSD: 1.3%	RSD: 1.1%

Table 4 Parameters of HPLC method and validation for hydroquinone and kojic acid in skin lightening products

Skin lightening products containing hydroquinone and kojic acid Method (adopted from Wang *et al.* 2015)		
Column	Agilent Zorbax Eclipse XDB-C18 4.6 × 250 mm (5 μm) with LiChrospher 100 RP-18 (5 μm) guard column	
Mobile phase	2 parts methanol; 23 parts deionized H_2O (0.1% acetic acid)	
Flow rate	0.5 ml/min	
Wavelength	280 nm	
Sample preparation	0.5 g drug product and 8 ml extraction media (9 parts 20 mM phosphate buffer pH 2.3/1 part methanol) were transferred to 15 ml centrifuge tubes. Sonication for 30 min and centrifugation for 30 min (1664*g*), repeated twice. Withdrawal of the clear solution and addition of 2 ml extraction media to the semisolid residue. Repetition of sonication and centrifugation. Combination of extract solutions, filter through 2.7 μm glass fiber filter and afterwards through 0.45 μm PTFE filter into 10 ml volumetric flask. Make up volume to 10 ml with extraction media, analyse content with HPLC UV analysis	
Validation	**Hydroquinone**	**Kojic acid**
Reference standard	Hydroquinone Sigma-Aldrich (Germany) Secondary Standard PHR1469-1G, Lot: LRAA9224	Kojic acid Sigma-Aldrich (Germany), Analytical Standard 95197-100MG, Lot: BCBS0131V
Retention times (min)	9.9 min	8.3 min
Peak symmetry	1.3	1.8
Resolution	Resolution between hydroquinone and kojic acid: 3.9	
Calibration curve	y (peak area) = 176 021x (μg/ml) − 1256	y (peak area) = 397 258x (μg/ml) − 49 512
Calibration curve range	5 30 μg/ml	5 30 μg/ml
Correlation coefficient	0.9997	0.9999
LOQ	<0.7 μg/ml	<1.9 μg/ml
Recovery	103.9 ± 4.74%	80.1 ± 9.5%
Repeatability	RSD: 0.92%	RSD: 3.1%
Reproducibility	RSD: 2.4%	RSD: 3.4%

the box-whisker plot in Figure 2 show that the results of the product sample obtained from the wholesaler are in accordance with the results from the products obtained in pharmacies: the South African product Amoxiclav releases significantly less amoxicillin within 30 min than the corresponding German product.

The differences observed in the USP quality control dissolution test were also seen in the dissolution test at pH

Table 5 Parameters of HPLC method and validation for glucocorticoids in skin lightening products

Method (adopted from Gaudiano *et al.* 2010)		
	Clobetasol diproprionate	Betamethasone dipropionate
Column	Agilent Zorbax Eclipse XDB-C18 4.6 × 250 mm (5 μm) with LiChrospher 100 RP-18 (5 μm) guard column	Merck Purospher STAR RP18e 4.6 × 250 mm (5 μm) with LiChrospher 100 RP-18 (5 μm) guard column
Mobile phase	10 parts methanol, 42.5 parts $NaH_2PO_4 \cdot H_2O$ solution pH 5.5, 47.5 parts acetonitrile	60 parts acetonitrile, 40 parts deionized H_2O
Flow rate	1.5 ml/min	1.5 ml/min
Temperature	25°C	30°C
Wavelength	240 nm	239 nm
Sample preparation	10 ml methanol was added to 0.6 g product. Samples were vortexed, sonicated and centrifuged (1664*g*, 10 min). 0.75 ml were withdrawn and diluted to 1.5 ml with mobile phase and measured via HPLC	10 ml methanol was added to 0.5 g product. Samples were vortexed, sonicated for 80 min and centrifuged (1664*g*, 10 min). 1 ml was withdrawn and measured via HPLC
Validation		
	Clobetasol diproprionate	Betamethasone dipropionate
Reference standard	Clobetasol propionate Sigma-Aldrich (Germany) Analytical Standard C8037-100MG, Lot: SLBD3909V	Betamethasone diproprionate Sigma-Aldrich (Germany), Pharmaceutical Secondary Standard PHR1399-1G, Lot: LRAA9185
Retention times (min)	14.5 min	13.6 min
Peak symmetry	1.04	1.12
Resolution	2.2 between clobetasol dipropionate and impurity D	11.8 between betamethasone valerate and betamethasone dipropionate
Calibration curve	*y* (peak area) = 85 496 435.5*x* (mg/ml) − 64 049.7	*y* (peak area) = 72 230*x* (μg/ml) + 12 940
Calibration curve range	10.5 19.5 μg/ml	20 70 μg/ml
Correlation coefficient	0.999	0.9998
LoD	<1.2 μg/ml	<0.03 μg/ml
LoQ	<0.4 μg/ml	<0.09 μg/ml
Recovery	89.2 ± 2.7%	94.4 ± 1.3%
Repeatability	RSD: 0.11%	RSD: 0.24%
Reproducibility	RSD: 1.15%	RSD: 7%

Table 6 Results of quality dissolution test according to USP indicating the mean percentage released of labelled API

Test level	Batch	German product			South African product
S1	Amoxicillin	100.7	99.3	101.0	86.6
N = 6	Clavulanic acid	98.1	100.9	97.4	98.7
S2	Amoxicillin	Not necessary			86.7
N = 12	Clavulanic acid				99.0

API, active pharmaceutical ingredient; USP, United States Pharmacopiea.

6.8. Although clavulanic acid is very rapidly dissolving (≥85% release in ≤15 min) in SIF_{sp} for both drug products, more than 85% amoxicillin is released within ≤15 min from the German product, which can therefore be characterized as 'very rapidly dissolving' with respect to amoxicillin. The South African product, however, takes 30 min to release more than 85% amoxicillin and can therefore be characterized as only 'rapidly dissolving' (Figure 3).[31]

Mometasone furoate

With respect to nasal sprays containing mometasone furoate as the active ingredient, the South African product also exhibited differences in quality compared to the German product. The German nasal spray passed the pharmacopoeial content uniformity test at stage one with a mean release of 106.4% of the labelled amount, a standard deviation of 3.1% and an acceptance value of 12.4%. The South African counterpart failed not only stage one but also stage two testing as the mean spray dose content was 113.7% of the labelled amount, and with a standard deviation of 6.5%, the acceptance value was calculated to be 27.9%, substantially higher than the cut-off value of 15%.

Skin lightening products

Table 7 depicts the assay results for the skin lightening products. The qualitative composition of the skin lightening agents of all but one product investigated coincided

with the labelling on the drug product. The exception was product (7), in which no kojic acid was detected. This discrepancy is likely due to the fact that the analytical method used is not appropriate for detecting and quantifying derivatives of kojic acid such as the dipalmitate ester, which differs from kojic acid in its physicochemical properties. For two products, a significantly higher percentage of hydroquinone was detected than had been declared on the label. In products (1) and (2), hydroquinone contents of $3.51 \pm 0.15\%$ and $2.83 \pm 0.12\%$ were measured as opposed to the declared amount of 2.0%. Data analysis via Student's *t*-test with Bonferroni's correction demonstrated a statistically significant, higher amount of hydroquinone in both of these products than was declared on the label ($P < 0.0167$). The amount of $2.14 \pm 0.21\%$ hydroquinone measured in product (5) was in agreement with the labelled amount. The qualitative and quantitative composition of the products containing corticosteroids were generally in good agreement with the label claim, although the measured content of the semisolid dosage forms (products 9–11) was slightly lower than the label claim. The lower values in the creams can be explained by incomplete extraction, as can be seen in Table 5, where recovery from the creams was found to be about 90–94%.

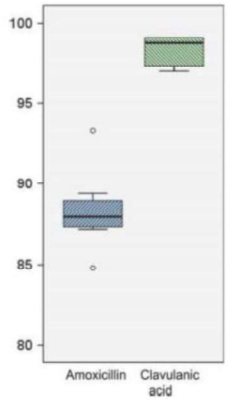

Figure 2 Dissolution tests results from a former study performed with Auro Amoxiclav 625 collected in South Africa 2016.[24]

Visual inspection of the confiscated skin lightening products revealed several conspicuous features, especially when compared to the products legally obtained from the German market, which showed no irregularities and were all labelled as 'bleaching product without hydroquinone'. The products that contained unusually high amounts of hydroquinone (products (1) and (2)) also showed the most obvious irregularities in their labelling. Both confiscated products exhibit an empty field where a NAFDAC (National Agency for Food and Drug Administration and Control, Nigeria) registration number is to be inserted. Furthermore, the label of product (1) is misleading: the formulation is an oil, even though the label asserts that the product is a cream, 'UVA, UVB' is included on the excipient list, and the lot number is unreadable. In addition to the labelling irregularities, the pharmaceutical quality of the formulation of product (2) was questionable. After opening the sealed container, widespread crystal growth was visible on the surface of the semisolid formulation. A pilot assay of samples consisting mostly of the crystallized substance resulted in hydroquinone contents of up to 17%. Furthermore, the content was observed to reside at a tilted angle in the primary packaging material, suggesting inappropriate product storage.

Product (5), while complying with the qualitative and quantitative composition stated on the label, also showed abnormalities on the label and in the formulation itself.

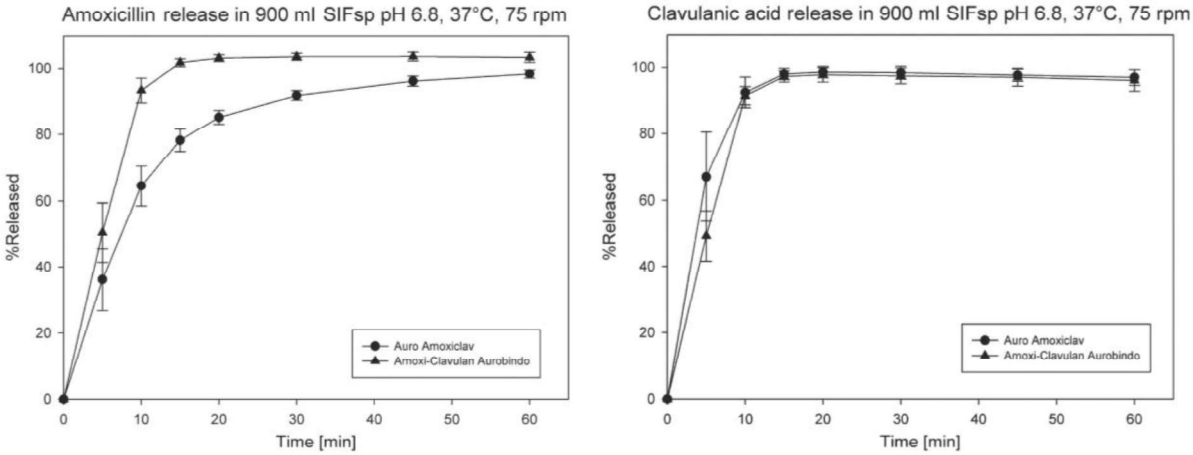

Figure 3 Release profiles for the German (Amoxy-Clavulan Aurobindo) and South African (Auro Amoxiclav) Amoxiclav products ($n = 5$, error bars depict standard deviations).

Table 7 Results of the quantitative analysis of the skin lightening products

Skin lightening product	Content hydroquinone measured	Labelled content hydroquinone	Content kojic acid measured	Labelled content kojic acid
1) Carotone oil	3.51 ± 0.15%	2%	0.077 ± 0.006%	Declared on label
2) Carotone black spot corrector	2.83 ± 0.12%	2%	0.023 ± 0.001%	Declared on label
3) So white skin perfector cream	2.773 ± 0.023%	Declared on label, without indication of quantity	None detected	N/A
4) So white lait skin perfector	3.38 ± 0.29%	Declared on label, without indication of quantity	None detected	N/A
5) Clear essence medicated fading cream	2.14 ± 0.21%	2%	None detected	N/A
6) Carotone brightening body lotion	None detected	N/A	1.05 ± 0.11%	Declared on label
7) So white skin perfector gel	None detected	N/A	None detected	Declared on label (as dipalmitate ester)
8) Clear essence medicated cleansing bar plus exfoliants	None detected	N/A	None detected	N/A

Skin lightening product	Clobetasol dipropionate measured	Labelled clobetasol dipropionate	Betamethason dipropionate measured	Labelled betamethason dipropionate
9a) Lemonvate cream 1592039	0.045 ± 0.001%	0.05%	None detected	N/A
9b) Lemonvate cream 1592038	0.046 ± 0.006%	0.05%	None detected	N/A
10) Movate cream	0.044 ± 0.005%	0.05%	None detected	N/A
11) Funbact-A triple action cream	None detected	N/A	0.059 ± 0.002%	0.0643%
12) Epiderm lotion	None detected	N/A	0.066 ± 0.002%	0.0643%
13) Betavate gel	None detected	N/A	None detected	N/A

N/A no information available on the label.

The same manufacturer is indicated on both the German and South African products. However, the German product (8) exhibited a different hologram when compared to the South African product (5). In addition, while major parts of the formulation of product (5) can be described as a homogenous white cream, there was visible black/brown discoloration of this product close to the lid, indicating that oxidation of the product had occurred.

Discussion

Over the last decades, the number and size of globally acting pharmaceutical companies has increased. It is common for world leading generic drug companies to produce products for a variety of different markets. The question arises, as to whether quality standards remain consistent for a given product, irrespective of where it will be marketed.

The results of this study suggest that the two Indian companies, whose products were analysed, manufacture prescription medicines that differ in quality for the German compared with the South African market. The authors chose the analysed prescription products on the basis that both companies act globally, are among the largest generic manufacturers and market the same products in both Germany and South Africa. It should be noted that other studies, such as the one published by Löbenberg *et al.* about products marketed in South America, show that such discrepancies are not at all limited to Indian companies.[32] Further investigations will be necessary to evaluate how widespread discrepancies in product quality are from country to country.

In our first case study, in which tablets containing amoxicillin and clavulanic acid (by Aurobindo) were evaluated, the German product passed all pharmacopoeial quality tests at the first level. Albeit showing similar results for the content uniformity test in compliance with the pharmacopoeial requirements, the South African product differed with respect to dissolution testing. For a comparison of the dissolution profiles, SIF_{sp} was chosen as the test medium. It has a pH of 6.8, corresponding to conditions in the proximal small intestine. The release of amoxicillin is slower in this medium than at lower pH values, and it is therefore considered more suitable for detecting differences between drug products.[32,33] Furthermore, degradation at the pH of SIF_{sp} (6.8) is negligible compared to degradation in more acidic media, eliminating a potential source of variability in the results.[34] The product marketed in Germany shows a faster release of amoxicillin than the South African product in SIF_{sp}. No comparison via an f_2-test was possible in this case, as the product marketed in Germany reaches 85% release in less than 15 min, precluding the availability of sufficient time points for a valid f_2-test comparison of the profiles. Although the dissolution profiles differ, an influence on the therapeutic efficacy seems unlikely. However, the slower release of amoxicillin from the South African product could potentially lead to a slight increase of gastrointestinal side effects

such as diarrhea, which can occur if unabsorbed amoxicillin reaches distal intestinal compartments and interacts with the intestinal microbiota.[35] Several possible explanations for the slower release of amoxicillin can be put forward, including differences in particle size, API distribution in the tablet, formulation and/or production parameters.

The results of the second case study, mometasone furoate nasal spray (by Cipla), indicate significant differences in quality among the products. While the German product passed the content uniformity test at the first level, the South African version of the nasal spray failed both stages, in terms of content uniformity. Consequently, the South African nasal spray is categorized as a poor quality product.

In October 2017, we informed Cipla, the manufacturer, about the results. Despite multiple phone calls and mails, no final explanation was presented and it is assumed that the company is still investigating the reasons for the failures (as of March 2018). Three explanations for the poor quality of the nasal spray product can be postulated. First, an error during production could have led to a higher concentration of the API in the solution. This would explain the mean dosage of 113.7% label claim, but not the high standard deviation. The second, and perhaps more likely, explanation could be a defect in the dosing mechanism of the primary packaging. This would explain not only the higher average content per metered dose but also the high standard deviation. Third, inappropriate or faulty analytical methods used by the manufacturer or in our study could have led to inappropriate acceptance values. As the method used to generate the results in this study was validated according to ICH guidelines, a calibration curve was performed every day prior to analysis, and experiments were performed in triplicate, this explanation appears to be the least probable.

The question arises, why companies would produce prescription medicines of different pharmaceutical quality for Europe and Africa. The authors of this study would like to offer four possible explanations:

First, the historical development of expansion into foreign markets could be a root cause of the variations. If a manufacturer launches its product in countries with less stringent regulatory requirements and then expands into new markets, it may well encounter higher quality standards that need to be met. Additionally, as experience and expertise regarding the formulation and manufacturing process is acquired, better quality products can be made. As a consequence, the product might be expected to exhibit higher quality in more recently entered markets than in older ones. This hypothesis is supported by the approval dates for mometasone furoate nasal spray by Cipla (GER: Dec 2014,[36] SA: Aug 2006 [37]) and Amoxiclav by Aurobindo (Ger: Jul 2010,[38] SA: Aug 2008[39]).

Second, differences in drug product quality may arise from differences in reference materials specified by national regulatory authorities as comparators. For example, differences in quality among pharmaceutically equivalent products from the same manufacturer were also observed in a study by Löbenberg *et al.*[32] In that case, two 500-mg amoxicillin drug products, one marketed in Argentina, the other one in the USA, manufactured by the same pharmaceutical company, showed dissimilar release *in vitro*. The manufacturer explained the discrepancy on the basis that the products were tailored to be bioequivalent with different reference products, depending on the country where market approval was sought.

Third, the company's financial interests also have to be taken into consideration. High-quality facilities and raw materials as well as top-drawer operational and quality control equipment are expensive. Lowering the quality standards to an extent that still complies with local regulatory requirements during production could lead to savings and higher profits. If regulatory inspections are few and infrequent, a manufacturer might be tempted to push the envelope even further.

As a fourth hypothesis, the results could also have been a coincidence. Other products or batches might have led to results indicating that there are no quality differences among products associated with where they are marketed. It would be necessary to study a much wider range of products and countries to determine how widespread the issue is.

Notwithstanding, it is interesting to compare the public health policies in Germany and South Africa: South Africa is one of the leading economies in Africa but struggles with impending economic recession caused by political instability.[40,41] As a result, the resources available for public health care in South Africa differ significantly from Germany (Table 8). It is therefore no surprise that the current regulatory agency for medicinal products in South Africa, the Medicine Control Council (MCC), has significantly fewer human resources available than the German authorities. At the moment, South Africa is implementing a new agency (South African Health Products Regulatory Agency). This agency will have considerable more employees than the MCC (about 400 vs 100),[42] but even then it will still correspond to only a fraction of its German counterpart.

In Germany, two different agencies are responsible for approval and monitoring of medicines. Together these agencies, the Federal Institute for Drugs and Medical Devices (BfArM) and the Paul-Ehrlich-Institut (PEI), employ approximately 1900 people.[43,44] These resources are supported on the European level by those of the European Medicines Agency (EMA). Additionally, each federal state in Germany and in South Africa has its own regional

Table 8 Economic and health indicators for Germany and South Africa

	Germany	South Africa
Population (million)[50]	82.67	55.91
GDP (billion US$)[51]	3466.76	294.84
Health expenditure (% of GDP)[52]	11.3	8.8
Life expectancy (years)[53]	81.0	62.9
Population privately insured (%)[54,55]	10.6	17

councils to monitor the supply chain of pharmaceutical products.

Although the legal framework in Germany and South Africa are quite similar, stringent law enforcement is lacking in South Africa. Through interviews with a former MCC investigator, the authors of this study were able to gain an impression of the procedures of the MCC law enforcement unit. A pronounced dissatisfaction regarding the current situation was expressed. In the case of illegal medicines, most court cases are dropped without convictions or not even initiated at all. Primary problems named were understaffed institutions and the lack of specialized public prosecutors. The comparison of statistics in Table 8 confirms this subjective impression. Additionally, the MCC has limited executive competences and has to forward all cases to the police.

This lack of power could also be the explanation for the infiltration of large numbers of illegal skin lightening products in South Africa.[25,45] While the skin lightening products legally obtained in Germany for this study were all free from corticosteroids and hydroquinone, the products confiscated in South Africa contained substances that are not permitted in products that can be sold without prescription because of possible hazards for the public (which was the reason for their confiscation).[46] In particular, products containing hydroquinone are a high risk for consumers, as their visual appearance does not convey to unsuspecting customers that they are products which contain a highly potent, dangerous substance. Even more dangerous for the consumer is the fact that the declared amount on the label sometimes misrepresents the actual amount in the formulation, which is often higher than the amount declared. This was true for two of the products investigated (see Table 7), and similar results have been reported in a study by another research group.[47] Indeed, for some products, the content of hydroquinone was even higher than that allowed for approved prescription medicines (maximum of 2%).[46]

It appeared that most of the skin lightening products obtained from South Africa were produced for other markets. This assumption is based on the facts that on some packages, a NAFDAC number was found which is provided by the 'Nigerian Agency for Food and Drug Administration and Control' and that a second South African language (in addition to English) was missing from the packages. Additionally, none of the manufacturers responded to emails sent to the addresses provided and it was impossible to trace back the supply chain of the products. Consequently, the authenticity of the products is questionable – they may have been counterfeit. This would explain differences between the German and South African skin lightening products. If not, then the question arises why the same companies are manufacturing products complying with pharmaceutical and cosmetic laws for some, but not for all countries where the products are sold.

Irrespective of the root cause of the poor quality of some products, strong law enforcement, thorough analysis and an increased number of inspections are ways in which unintentional as well as deliberate supply of poor quality medicines can be prevented. Some key areas that should be addressed include:

1) A separate and specialized law with high penalties for counterfeit medicines should be drafted.
2) Customs officers should be taught how to distinguish illegal and legal medicines, in order to recognize not only trademark infringements of known brands, but also to prevent infiltration of genuine looking but harmful medicinal products.
3) The number of governmental law enforcement officers working in this area should be increased. Specialized police departments would be especially helpful.
4) More frequent inspections of supermarkets and stores that are suspected of selling illegal medicines should be made
5) Public education and availability of information regarding risks of illegal products and how to identify them should be improved.

Of course, all of these suggestions for improvement would need considerable funding. One possibility for countries with limited financial resources would be to increase taxes or approval fees for medicines. As these additional costs would likely increase drug prices, the ramifications for cost-benefit ratios would have to be taken into consideration.

Conclusion

Although only a limited number of samples were analysed, quality differences among pharmaceutical products ranging from slight to severe were identified. These discrepancies among products, even when produced by the same manufacturer, raise issues beyond ethical aspects. Regarding generics, more transparency for the consumer is necessary. Globally accepted reference products, such

as the products proposed in the WHO prequalification of medicines, could help to align the quality of generics worldwide. Countries like South Africa that struggle with a high incidence of poor quality drugs will need to re-evaluate their current legislative and executive means to ensure drug quality and to preclude those drug products with poor quality from entering their markets. Further scientific field studies and evaluations are necessary in other developing countries to evaluate the incidence of poor quality drugs and, where necessary, to determine which regulatory and legislative efforts are necessary to sustain a well-functioning public health system.

Declarations

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Suitability of the *z*-Factor for Dissolution Simulation of Solid Oral Dosage Forms: Potential Pitfalls and Refinements

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ABSTRACT

Parameterization of dissolution profiles for subsequent use in *in silico* modeling and simulation is a crucial element for the success of extrapolating *in vitro* to *in vivo* release from solid oral dosage forms. The z-factor dissolution model is an option that can be utilized in commercial software such as GastroPlus™ to simulate the release from solid oral dosage forms. However, several aspects that can confound particle dissolution, such as disintegration and coning, are currently not taken into consideration in this model. To promote a more comprehensive use of the z-factor dissolution model, we discuss the scope of the model in its current modus operandi, highlight problems associated with the current approach and present potential solutions. Taking into account disintegration of dosage forms together with a calculation of the theoretical mass available for dissolution allows for a more realistic z-factor estimate that considers the dissolution process in terms of its two core components, dosage form disintegration and particle dissolution, independently. It is shown that separating these two elements allows for more flexible evaluation and use of the z-factor approach in modeling softwares, as both elements can then be scaled independently to describe the behavior in a range of simulated physiological environments.

Introduction

Physiologically based biopharmaceutical modeling and simulation approaches are becoming more frequently applied at early stages of drug formulation development as well as during life cycle management of approved drug products, e.g. for justification of new quality control test specifications in the course of scale up and post approval changes (SUPAC).[1] For drugs formulated in solid oral dosage forms, the dissolution process and the parameterization of experimental *in vitro* data for later use in *in silico* modeling approaches is a crucial aspect that can have a major impact on the success of predicting the performance of a drug product *in vivo*. Theoretical models have been developed that either describe the dissolution process empirically or use a mechanistic approach that is, in most cases, based on variations of the dissolution equation derived by Noyes and Whitney.[2] One of the variations is the *z*-factor equation described by Nicolaides et al.[3] in 2001 and Takano et al.[4] in 2006. It is a simplified mechanistic model that assumes a spherical particle shape and uniform particle size for dissolution. The z-factor can be utilized as an input option in the commercial modeling software GastroPlus™ and has been used with varying degrees of success in several recent publications to assess the potential impact of the dissolution behavior, as determined in *in vitro* studies, on pharmacokinetic outcome parameters.[1,5–7]

One source of problems encountered when fitting the *z*-factor model to observed dissolution data is that the equation considers the whole dose of a substance to be immediately available for dissolution, which is an oversimplification if the disintegration time is significant, as is the case for many solid oral dosage forms. Another factor that can reduce the amount of drug substance available for dissolution when using compendial dissolution testers (e.g. the USP II apparatus) is the occurrence of coning, which results in a fraction of the drug being unavailable for dissolution due to being 'trapped' in a zone with poor hydrodynamics.

In order to promote a more rational use of the *z*-factor to describe the *in vitro* dissolution behavior of active pharmaceutical ingredients (APIs) formulated in solid oral dosage forms, we discuss the scope of application of the *z*-factor equation, highlight

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problems on the basis of theoretical and practical examples, and propose an approach for a more realistic estimate of the z-factor that can also be used to determine the validity of results obtained with usual methods employed to calculate the z-factor from experimental dissolution data.

Assumptions of the z-Factor Dissolution Equation

The z-factor dissolution equation is based on the dissolution equation established by Noyes and Whitney[2] (Eq. (1)), which defines the dissolution rate $\frac{dM_s(t)}{dt}$ of the mass $\boldsymbol{M_s}$ of undissolved particles in a medium as being dependent on the diffusion coefficient $\boldsymbol{D}$ of the API in the media, the total surface area of the undissolved particles $\boldsymbol{A}$ at time $\boldsymbol{t}$, the length of the hydrodynamic diffusion layer $\boldsymbol{h}$, the saturation solubility of the API $\boldsymbol{C_s}$ and the API concentration $\boldsymbol{C}$ at time $\boldsymbol{t}$.

$$-\frac{dM_s(t)}{dt} = \frac{D \cdot A(t)}{h} \cdot [C_s - C(t)] \tag{1}$$

Assuming all particles to be of spherical shape with the same initial particle radius $\boldsymbol{r_0}$, the relationship between the initial particle mass $\boldsymbol{M_0}$ and the undissolved particle mass $\boldsymbol{M_s}$ (where $0 \leq \boldsymbol{M_s} \leq \boldsymbol{M_0}$) may be used to describe the surface available for dissolution instead of the particle radius. Considering the spherical volume to surface ratio $\frac{r_0}{3}$ and uniform particle density $\boldsymbol{\rho}$, Equation (1) may be transposed to:

$$-\frac{dM_s(t)}{dt} = \frac{3D}{h \cdot \rho \cdot r_0} \cdot M_0^{\frac{1}{3}} \cdot M_s(t)^{\frac{2}{3}} \cdot [C_s - C(t)] \tag{2}$$

Assuming the ratio $\frac{D}{h}$ to be constant during the dissolution process. the dissolution factor $\boldsymbol{z}$ is introduced as a hybrid parameter $\frac{3D}{h \cdot \rho \cdot r_0}$ such that

$$-\frac{dM_s(t)}{dt} = z \cdot M_0^{\frac{1}{3}} \cdot M_s(t)^{\frac{2}{3}} \cdot [C_s - C(t)] \tag{3}$$

When sink conditions are additionally assumed (usually when $\boldsymbol{C_s}$ is at least 3 times higher than the observed $\boldsymbol{C(t)}$ for all data points), the equation is simplified even further:

$$-\frac{dM_s(t)}{dt} = z \cdot M_0^{\frac{1}{3}} \cdot M_s(t)^{\frac{2}{3}} \cdot C_s \tag{4}$$

As described by Nicolaides et al.,[3] the differential equations (Eqs. (3) and (4)) can be integrated and fitted to the experimental dissolution data in order to obtain an estimate of the z-factor describing the dissolution process. Equation (5) depicts the integral of Equation (4). This equation is used throughout this manuscript to obtain what we define here as the 'traditional z-factor estimate' under the assumption of sink conditions.

$$\frac{3 \cdot M_s(t)^{\frac{1}{3}}}{M_0^{\frac{1}{3}} \cdot C_s} + \frac{3}{C_s} = z \cdot t \quad | \; for: \; t \leq \frac{3}{z \cdot C_s} \tag{5}$$

The traditional z-factor estimate is obtained from the slope of the linear regression equation for a plot of the left hand side of Equation (5) against $\boldsymbol{t}$.

Potential Problems Associated With the Application of the Traditional z-Factor Equation

Conventionally, in the calculation of a z-factor, $\boldsymbol{M_0}$ represents the complete dose of an API used in the dissolution experiment. This implies that the complete dose of an API is immediately available for dissolution. The dissolution rate $\frac{dM_s(t)}{dt}$ therefore has its highest value at t = 0 and continuously decreases as the amount of solid mass $\boldsymbol{M_s}$ available for dissolution decreases. However, many solid oral dosage forms exhibit a sigmoidal dissolution profile shape due to an initial disintegration process. For such dosage forms it is not appropriate to fit the data using the traditional z-factor approach. Strictly speaking, the z-factor equation is only suitable for drug products that have no significant disintegration time and for which the assumption of the complete mass being immediately available is therefore reasonable. This may be the case for drugs formulated as powders, suspensions and possibly even granules, but is to be critically reviewed for solid oral dosage forms such as tablets and capsules where the mass available for dissolution at early time points is often influenced by disintegration.

In a similar manner, the occurrence of coning can reduce the amount of drug available for dissolution, with the greatest effect on concentrations measured at late dissolution time points. Both phenomena may lead to an underestimate of true particle dissolution rate since in the z-factor model, the dissolution rate is driven by the z-factor and the solid mass available for dissolution (Eq. (4)). Thus, if the dissolution rate $\frac{dM_s(t)}{dt}$ is interpreted using Equation (4) under the assumption that the entire solid mass is available for dissolution, whereas in fact slow disintegration and/or coning limit the amount of solid drug available for dissolution, an underestimate of the magnitude of the z-factor will result.

Several examples can be found in the recent literature in which the z-factor equation is used to parameterize experimental dissolution data even though coning is observed and the disintegration time is substantial.[5,7] Therefore, there is a need to point out the importance of a thoughtful assessment of whether a given set of experimental data can be adequately described by traditional application of the z-factor equation.

A potential solution for obtaining a realistic z-factor estimate when dissolution is influenced by disintegration and/or coning is described in the following section. The beneficial aspects of the approach are exemplified with experimental data and are then further substantiated with a theoretical case study as well as case examples of experimental data taken from the recent literature.

Theory and Application Example

Estimation of the Available Mass Range

Once a z-factor estimate is obtained from fitting the dissolution data to Equation (5), it is prudent to assess its suitability to describe the observed *in vitro* dissolution profile. Using the method proposed below, the range of the mass of solid drug substance that could be available for dissolution can be calculated from the slope of the release profile. For simplicity, sink conditions are assumed here. However, this approach can also be adapted to non-sink conditions. Experimentally, reliable data for the slope at each time point can be obtained by either using real-time measurement of the drug concentration in the media (e.g. using fiber optics or devices such as the µDISS) or with a high sampling frequency over the expected duration of disintegration and/or dissolution time. In order to obtain an estimate of the dissolution rate at each time point, the experimental dissolution data may be described empirically by Weibull/RRSBW distribution functions[8] (Eq. (6)). The dissolution rate at each time point is estimated from the derivative of the Weibull function (Eq. (7)):

$$R(t) = Max \cdot \left(1 - f_1 \cdot e^{\left[\frac{-(t-T)^{B_1}}{A_1}\right]} - f_2 \cdot e^{\left[\frac{-(t-T)^{B_2}}{A_2}\right]} \right) \quad (6)$$

$$\frac{dR(t)}{dt} = Max \cdot \left(\frac{f_1 \cdot B_1 \cdot (t-T)^{(B_1-1)} \cdot e^{\left[\frac{-(t-T)^{B_1}}{A_1}\right]}}{A_1} + \frac{f_2 \cdot B_2 \cdot (t-T)^{(B_2-1)} \cdot e^{\left[\frac{-(t-T)^{B_2}}{A_2}\right]}}{A_2} \right) \quad (7)$$

with: $\boldsymbol{R}$: Amount of dose released [%] $\boldsymbol{Max}$: Maximum amount of dose released [%] $\boldsymbol{f_{1,2}}$: Fraction factors $\boldsymbol{T}$: Time lag until start of dissolution [h] $\boldsymbol{t}$: Time [h] $\boldsymbol{B_{1,2}}$: Shape factors $\boldsymbol{A_{1,2}}$: Time scale factors.

For a given z-factor, the possible range of solid mass available for dissolution at each time point can then be calculated assuming two extreme scenarios:

1. Under one assumption, the slope of the dissolution profile arises solely from undissolved drug particles that still have their initial particle radius $\boldsymbol{r_0}$. This results in the maximum possible available solid mass $\boldsymbol{M_{sMax}}$ at a specific time point, as the surface area to volume ratio of a sphere $\left(\frac{Surface}{Volume} = \frac{4 \cdot \pi \cdot r^2}{\frac{4}{3} \cdot \pi \cdot r^3} = \frac{3}{r}\right)$ can only increase during the dissolution process of spherical particles of similar initial size. $\boldsymbol{M_{sMax}}$ is calculated with Equation (8).

$$M_{sMax}(t) = -\frac{dM_s(t)}{dt} \cdot \frac{1}{z \cdot C_s} \quad (8)$$

2. Under the other assumption, the slope of the dissolution profile arises from partially dissolved drug particles. Drug particles that are partially dissolved have a higher surface area to volume ratio compared to the initial particle radius and thus require a lower total volume (and thus a lower total particle mass) to create the same effective surface area. Assuming a uniform initial particle size of $\boldsymbol{r_0}$, the available solid mass $\boldsymbol{M_s}$ at any given time derives from a total initial mass $\boldsymbol{M_i}$ available for dissolution which has been reduced by the observed cumulative dissolved mass $\boldsymbol{M_d}$. The lowest possible value for solid mass available for dissolution $\boldsymbol{M_{sMin}}$ is obtained when $\boldsymbol{M_d}$ is assumed to be equally deducted from all initial particles, resulting in the radius of all particles being uniformly reduced from $\boldsymbol{r_0}$ to a certain smaller radius $\boldsymbol{r_1}$ (explanation is given in the Supplementary Material). Knowing $\boldsymbol{M_{sMax}}$ and $\boldsymbol{M_d}$ for a given time point, the initial mass available for dissolution $\boldsymbol{M_i}$ may be calculated from Eq. (9). $\boldsymbol{M_{sMin}}$ is then obtained by deducting the observed dissolved mass $\boldsymbol{M_d}$ from $\boldsymbol{M_i}$.

$$M_i(t)^3 - 2 \cdot M_d(t) \cdot M_i(t)^2 + M_d(t)^2 \cdot M_i(t) - M_{sMax}(t)^3 = 0 \quad (9)$$

where $M_i(t) - M_d(t) \geq 0$.

The total mass range that must have been available up to each time point during the dissolution process is obtained by adding the experimentally observed dissolved mass $\boldsymbol{M_d}$ to the calculated maximum and minimum available solid masses, respectively. Under the assumption that the mass that is available for dissolution cannot become unavailable during the dissolution process (i.e. that no precipitation or coning occurs), this range also serves as an estimate for the range of a theoretical disintegration profile for a given z-factor, as the cumulative disintegrated mass at each time point is the sum of the solid mass available for dissolution and the mass that has already been dissolved. Realistically, the available mass (dissolved and undissolved) cannot exceed the dose used in the dissolution experiment. Therefore, plotting the available mass range alongside the experimental dissolution data allows for a facile assessment of the plausibility of a fitted z-factor.

Application Example for the Available Mass Range Approach and the z-Factor Estimate

Fig. 1 depicts an experimentally obtained dissolution profile for Doxycyclin AL 200 T, a tablet formulation containing 200 mg of doxycycline (as doxycycline hyclate) in a USP II dissolution tester using 500 mL of Phosphate Buffer pH 2.7 and a rotational speed of 50 revolutions per minute (RPM). The product's qualitative composition and regulatory information can be found in the supplementary material (Table S1).

The saturation solubility of the API in this medium was measured to be 58.4 mg/mL and the median disintegration time of 3 tablets was 473s (at which time complete disintegration was observed visually). Using Equation (5), a traditional z-factor of 3.75×10^{-3} mL/mg/min was obtained. A Weibull function was fitted to the observed data and the available mass range for each time point was calculated as described in the previous section.

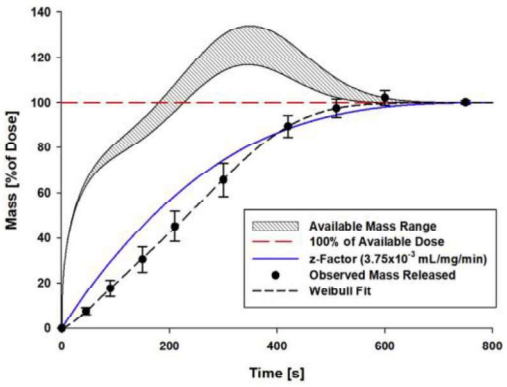

Fig. 1. Available mass range for an estimated z-factor obtained via traditional z-factor fit.

As can be seen in Fig. 1, available solid masses would be needed that (with the addition of the cumulative mass that has already been dissolved) exceed the dose used in the dissolution experiment at time points later than 200 s. Although the simulated particle dissolution profile solely based on the z-factor under consideration of the full dose (solid blue line) looks like an overestimate at first glance, it actually is an underestimate of the particle dissolution rate, as it is unable to realistically describe the dissolution rates observed after 200 s without applying solid masses that would, in addition to the mass already dissolved, exceed the dose used in the experiment.

In order to estimate a z-factor that is able to explain the observed dissolution profile more realistically, a refined z-factor z*(t) is calculated for each pair of $M_d(t)$ and $M_i(t)$. In this procedure, the minimum available mass is adjusted to a maximum value corresponding to 100% of the dose (Eq. (10)). The lowest possible z-factor that is able to realistically describe the observed dissolution profile is one for which the sum of $M_d(t)$ and $M_{sMin}(t)$ is $\leq$ 100% across the observed period of time. Thus, the minimum z-factor z_{min} is the highest z*(t) calculated from every pair of $M_d(t)$ and $M_i(t)$.

$$z^*(t) = z \cdot \frac{M_i(t)^{\frac{1}{3}} \cdot [M_i(t) - M_d(t)]^{\frac{2}{3}}}{Dose^{\frac{1}{3}} \cdot [Dose - M_d(t)]^{\frac{2}{3}}} \quad (10)$$

An example is illustrated in Fig. 2. The same observed dissolution data as in Fig. 1 is depicted, but here a calculated z_{min} of 6.32×10^{-3} mL/mg/min is used as the dissolution factor. It can be seen that the minimum available mass range never exceeds 100% of the dose, meaning that this new z-factor is the smallest particle dissolution factor able to explain the dissolution profile without the need for unrealistically high available masses.

An additional aspect that can now be taken into consideration is the experimentally observed disintegration time of the dosage form. If available, it can be used to verify or refine the range of available masses. In the exemplary dissolution profile shown in Figs. 1 and 2, the median observed time t_D until the disintegration is visually complete was 473 s (Range: 380s–490s). As can be seen in Figs. 1 and 2, the median disintegration time does not quite match the available mass range, as both examples show complete disintegration (i.e., 100% of the dose being available) prior to 400 s. For further refinement, the observed disintegration time can now be used to determine the range of possible z-values that is able to describe the dissolution profile while being in agreement with the observed disintegration times. The lower bound for the z-factor range is the z-value for which 100% available mass at t_D first enters the available mass range, and the upper bound is the highest z-value for which 100% available mass at t_D is still within the available mass range. For the exemplary dissolution profile, the boundary values of the range of possible z-factors are empirically found to be 6.32×10^{-3} and 1.72×10^{-2} mL/mg/min, respectively. This range can now be used to verify the traditional z-factor initially obtained via fitting Equation (5) to the experimental data. For this case example, it can be concluded that the traditional z-factor underestimates the particle dissolution rate by at least a factor of 2.

As the z-factor range calculated via the available mass range can be relatively broad depending on the mode of disintegration and the disintegration time in relation to the particle dissolution time, the range is of limited usefulness as input for *in silico* modeling of the dissolution process. A refined estimate of a z-factor can be obtained by adopting a numerical approach described by Nelson and Wang.[9] Using their approach, a theoretical disintegration profile can be calculated for a specific particle dissolution rate constant when sink-conditions can be assumed. In addition, as the theoretical dissolution profile is constructed on a consecutive basis from calculations of previous time points, it must be assumed that mass that has become available for dissolution cannot revert to the status of being unavailable. In order to obtain a smooth and accurate disintegration profile for a given z-factor, the time intervals for the numerical calculation have to be appropriately small. For the case example presented here, the theoretical disintegration profile is calculated using time intervals of 1s. An estimate of the z-factor is sought empirically for which the maximum/plateau of the calculated disintegration profile matches the observed disintegration time without the occurrence of negative particle masses. Negative particle masses can occur during the numerical calculation when the observed slope of the dissolution profile is smaller than the sum of the slopes created by the individual particles available for dissolution. In such cases, the value of the z-factor needs to be increased in order to avoid the need for negative particle masses. For the case example discussed here, it was empirically determined that a refined z-factor of 9.80×10^{-3} mL/mg/min matches the observed disintegration time and fulfills the conditions indicated above. Fig. 3 depicts the simulated particle dissolution profile based on the refined z-factor alongside the available mass range. This refined z-factor in combination with the theoretical disintegration profile is able to explain the observed dissolution profile as well as the disintegration time without the need for unrealistically high

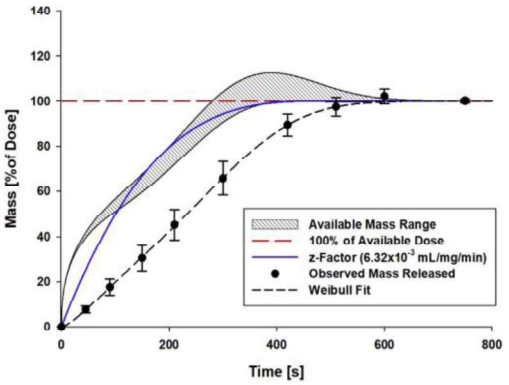

Fig. 2. Available mass range for the smallest value of a refined z-factor able to explain the observed dissolution profile.

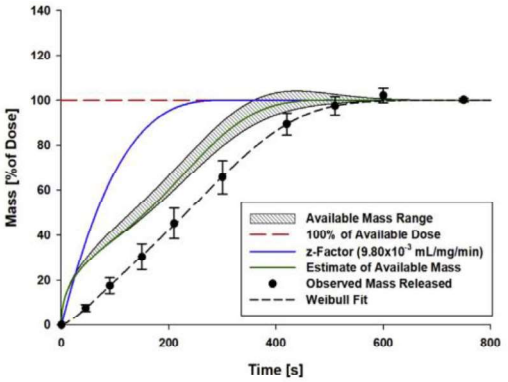

Fig. 3. Refined z-factor obtained from adopting the numerical approach described by Nelson and Wang[9] for calculation of a theoretical disintegration profile alongside the corresponding available mass range.

available masses. Furthermore, the numerical approach by Nelson and Wang gives a visual impression of what a theoretical disintegration profile for the given z-factor would look like. Both the refined z-factor and the calculated theoretical disintegration profile can subsequently be used as input parameters to describe the dissolution process in *in silico* simulations.

Hereinafter, in order to demonstrate the accuracy and usefulness of the described approaches for a refined z-factor estimate, both approaches are compared with the traditional z-factor fitting method on the basis of theoretical and practical examples. Lastly, case examples where coning was observed are discussed and advice is given on how such cases may be handled so that a suitable z-factor estimate may still be obtained.

Methods

Examination of the Influence of Disintegration on the z-Factor Estimate

In order to investigate the reliability of the traditional z-factor estimation and the proposed methods for refining the estimate, theoretical sets of dissolution profiles with disintegration times in the range of 0–600 s and different modes of disintegration behavior (linear disintegration, disintegration with a lag time, power function based disintegration, disintegration following a Weibull function) were generated based on a theoretical example of a highly soluble API with a dose of 500 mg, a theoretical z-factor of 0.17 mg/mL/min, a saturation solubility of 3.9 mg/mL and (resulting from the solubility and the theoretical z-factor), a particle life-span of 269 s. The calculated continuous dissolution profiles were divided into evenly spaced intervals in order to mimic sampled data points obtained from usual experimental procedures (Fig. 4). The resulting set of data points were subsequently used as a basis for the various z-factor estimation approaches.

The following approaches for z-factor estimation were used and compared according to the relative error $\frac{z_{estimated} - z_{theoretical}}{z_{theoretical}}$ of the z-factor estimate:

A) Application of the approach described by Nicolaides et al.[3]: The integral of the z-factor differential equation assuming sink conditions (see 'Theory' section, Eq. (5)) is fitted to the dissolution data with the following dissolution time points taken into consideration:

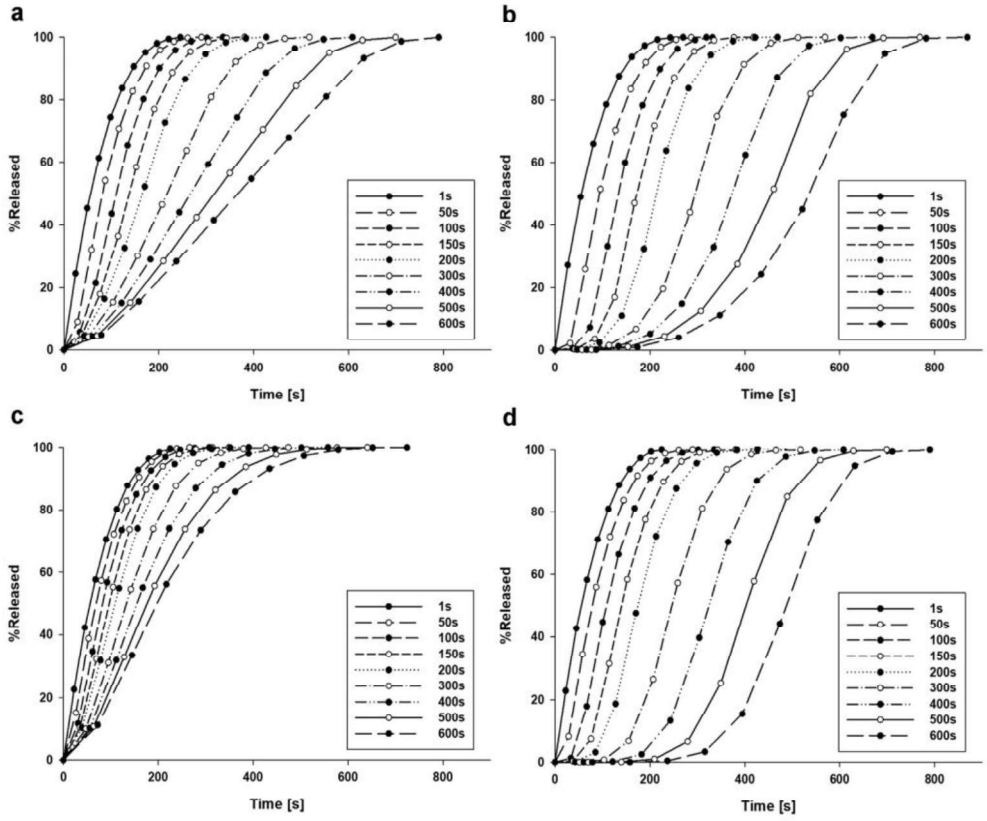

Fig. 4. Theoretical dissolution profiles calculated with disintegration times ranging from 0 to 600s with a disintegration mode of (a) linear disintegration (b) disintegration with lag time (c) power function based disintegration (d) disintegration following a Weibull function. Data points highlighted in the theoretical, continuous dissolution profiles were used for fitting the z-factor equation in the traditional z-factor estimation approach as well as for fitting a Weibull function in the refined z-factor estimation approach in order to resemble experimentally obtained data points from evenly distributed sampling times.

1. The whole dissolution profile (from t = 0 until the first time point where a plateau is reached indicated by a change in cumulative dissolved mass < 5%) is considered.
2. Fit through data points in vicinity of the inflection point of the dissolution profile curve (i.e. only data points with a change of ≥ 10% in cumulative dissolved mass compared to the previous data point are considered).
3. Assuming that the disintegration time is known, only data points after complete disintegration and until the plateau is reached are considered for the z-factor fit.

The same theoretical data set is also evaluated by estimation of the refined z-factor based on the theoretical available mass range approach described in the 'Theory' section of this manuscript (B) and the numerical approach described by Nelson and Wang (C):

B) Application of the available mass range approach:
   1. The minimum possible z-factor z_{min} that is able to explain the theoretical dissolution profile is estimated based on the observed dissolved mass and dissolution rate at each time point.
   2. Assuming the disintegration time to be known, the range of z-factors eligible to describe the profiles is estimated based on the observed dissolved mass and estimated dissolution rate at each time point.

C) Application of the numerical approach as described by Nelson and Wang[9]: An estimated disintegration profile is calculated based on the dissolved mass calculated from the fitted Weibull function. For this approach, only masses with physical meaning are considered (i.e. the disintegrated masses cannot be negative). The lowest z-factor that yields a disintegration profile that matches the observed disintegration time with a maximum/plateau in the range of 100 ± 1% of the API dose without the occurrence of negative particle masses is considered as z-factor estimate.

In addition to the theoretical case study, all approaches were applied to dissolution data taken from two recent publications in which traditional z-factors were used as input for *in silico* simulation of the dissolution process.[5,7]

Examination of the Influence of Coning on the z-Factor Estimate

Two case examples are discussed where the occurrence of coning during a dissolution test and the dose considered for the z-factor estimate have a noticeable impact on the suitability of the fitted z-factor. All dissolution tests were performed using the USP II apparatus with a rotational speed of 50 RPM. As dissolution medium, 500 mL of simulated gastric fluid without pepsin (SGFsp) pH 1.2 kept at a constant temperature of 37 ± 0.5 °C was used.

In the first case example, commercially available tablets (Amoxi-CT 750 mg) containing 750 mg amoxicillin (as amoxicillin trihydrate) were tested using regular vessels and peak vessels, respectively. A sample size of n = 6 tablets was tested with each vessel type. In contrast to the smooth, hemispherical bottom of regular dissolution vessels, peak vessels are provided with a small, upward pointing glass cone located at the center of the vessel bottom in order to prevent particles being trapped in a zone with poor hydrodynamics.

For the second case example, the individual dissolution results for two commercially available tablets (Doxy-m-ratiopharm 200 mg) containing 200 mg doxycycline (as doxycycline monohydrate) are discussed that were obtained from dissolution tests using regular vessels under identical conditions, where coning was observed for one drug product, but did not occur for the other tested product.

Both the amoxicillin and doxycycline drug products showed rapid and complete disintegration before the first sampling time point. Additional details such as the products' qualitative composition and regulatory information can be found in the supplementary material (Table S1).

Results and Discussion

Comparison of z-Factor Estimates Obtained From Theoretical Dissolution Profiles

The theoretical disintegration profiles used for the calculation of the dissolution profiles shown in Fig. 4 can be found in the Supplementary Material (Fig. S2). As can be seen in Fig. 5, irrespective of the mode of disintegration and time for complete disintegration, the traditional z-factor (obtained from fitting Eq. (5) to the observed data) greatly underpredicts the real z-factor used for generating the dissolution profiles when there is a marked disintegration time. Among the approaches examined, fitting the complete data set through origin yielded the worst results, while using data points near the inflection point and/or data points after the observed disintegration time yielded z-factor estimates that were still in reasonable proximity to the real z-factor when disintegration time was relatively fast compared to the particle dissolution time, e.g. when $\frac{Disintegration\ Time}{Dissolution\ Time} < 0.5$. When the influence of the disintegration process on the theoretical dissolution profile became more pronounced or even outweighed particle dissolution, the z-factor estimates obtained from the traditional method were afflicted with prediction errors as large as −80% for some theoretical case examples studied here.

In cases where the disintegration had noticeable impact on the dissolution profile, the calculation of the lower bound of the z-factor range resulted in smaller prediction errors compared to the traditional fitting method, but still clearly underpredicted the true z-factor, especially for longer disintegration times. Only when the disintegration time is known, a z-factor range could be defined that either included or, when disintegration time was relatively fast, slightly overpredicted the theoretical z-factor. However, the width of the obtained range was clearly dependent on the mode of disintegration, with the cases where disintegration was assumed to be linear or almost linear (with a preceding lag-time phase) resulted in narrow z-factor ranges (Fig. 5a and b), while disintegration behavior described by a power function (Fig. 5c) or a Weibull function (Fig. 5d) resulted in very broad z-factor ranges. Nonetheless, as the ranges include the true z-factor especially when disintegration is more pronounced, the approach is useful for verifying the plausibility of a traditional z-factor estimate.

In the case examples investigated, the smallest overall prediction errors were obtained adopting the numerical method described by Nelson and Wang[9] to find a z-factor estimate that resulted in a theoretical disintegration profile that matches with the observed disintegration time. However, the accuracy of this method showed a dependency on the mode of disintegration, as the obtained z-factor was an overestimate when the disintegration profiles were defined by an initial time lag (Fig. 5b) but was either in good agreement with the theoretical z-factor or slightly underestimated for the other disintegration modes studied, especially for higher disintegration times.

Exemplarily, the calculated disintegration profiles obtained from the Nelson and Wang method and the estimated z-factors for a disintegration time of 300s for all modes of disintegration considered here are depicted in the Supplementary Material (Fig. S3) and are compared to the original theoretical data sets.

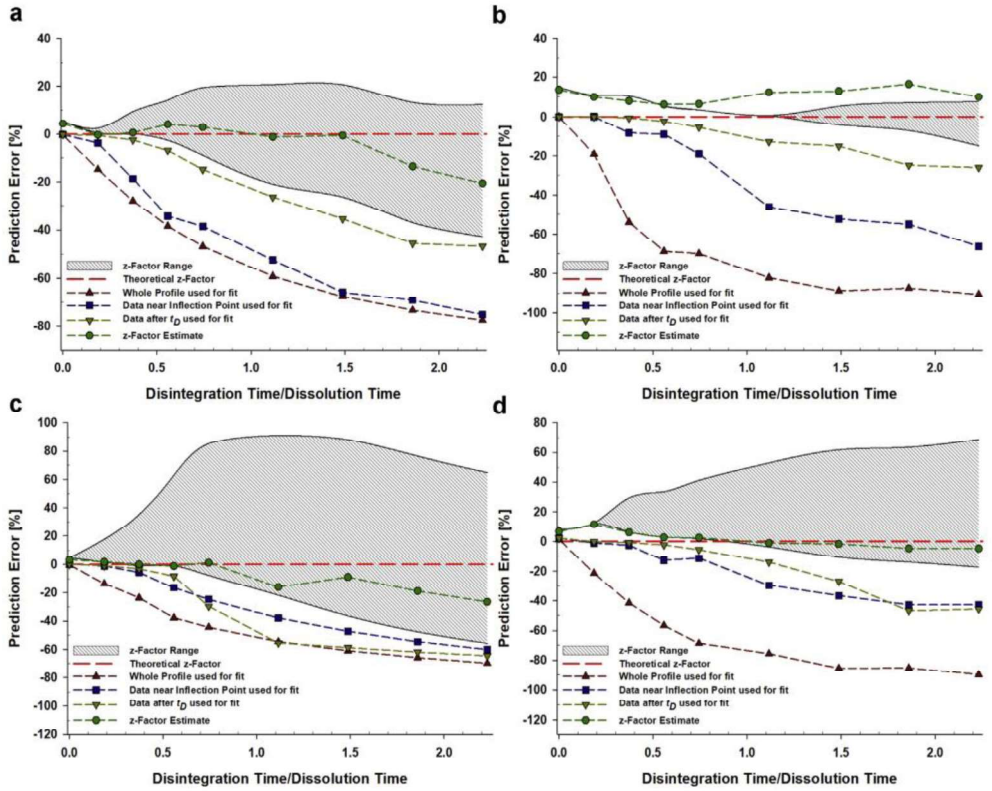

Fig. 5. Prediction error of the z-factor estimation approaches as a function of disintegration time relative to particle dissolution time for (a) linear disintegration (b) disintegration with lag time (c) power function based disintegration (d) disintegration following a Weibull function. In cases where the available mass range already surpassed the observed disintegration time even for the minimum z-factor, no upper bound for the z-factor range could be defined and is therefore not depicted.

Assessment of z-Factors Obtained From Experimental Dissolution Profiles

Case Example I: Experimental Data From Ding et al.[5]

In a study performed by Ding et al.[5], the dissolution process of the weak base galunisertib formulated in tablets was described using the z-factor dissolution model. Fig. 6a shows the dissolution profile of a tablet formulation in 0.01 N HCl from their study where the solubility of the drug was reported to be > 20 mg/mL and thus, sink conditions can be assumed for the dissolution of an API dose of 150 mg in 900 mL of media stirred with 75 RPM in the USP II apparatus. A disintegration time of 300s was reported. The dissolution data were taken from Fig. 3A of their original article.[5]

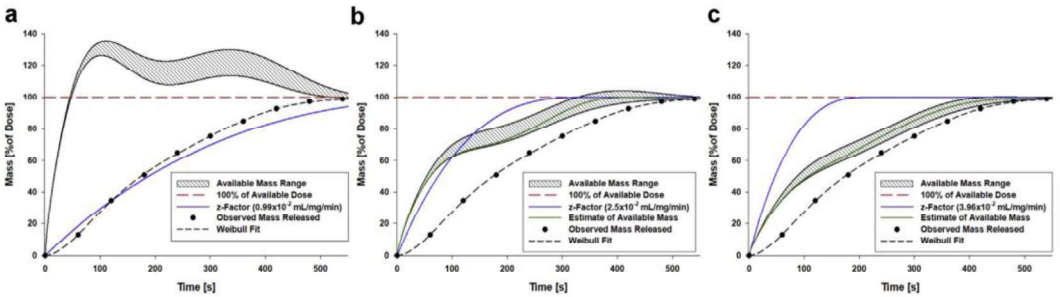

Fig. 6. Available mass range for dissolution data from Ding et al. for (a) the traditional z-factor fit (b) the refined z-factor estimate (c) the traditional z-factor increased by a factor of 4 as suggested by Ding et al.[5]

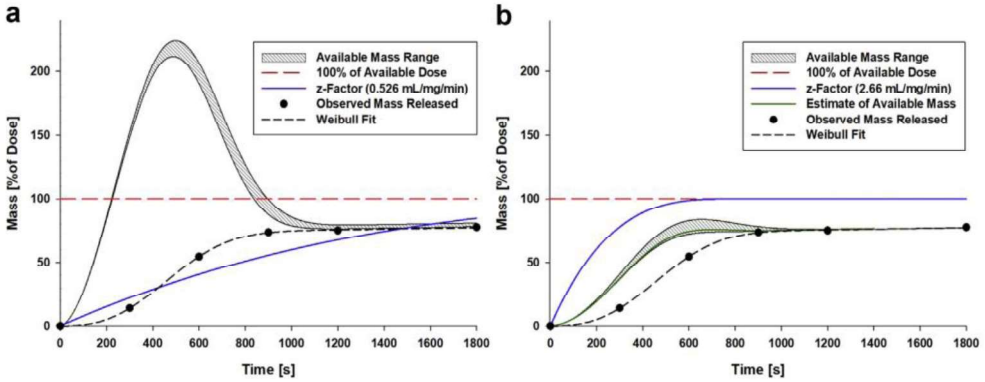

Fig. 7. Available mass range dissolution data taken from Li et al.[7] applying (a) the traditional z-factor fit (b) the refined z-factor estimate under consideration of the highest observed mass released as the maximum available dose.

Fitting a traditional z-factor to the experimental data, a value of 0.99×10^{-2} mL/mg/min is obtained and the corresponding simulated dissolution profile is depicted as the solid blue line in Fig. 6a. As can be seen from the calculated available mass range for this traditional z-factor, solid masses available for dissolution that exceed the dose used in the dissolution experiment would be needed. The mismatch between the experimentally observed disintegration time of 300s and the maximum value for the theoretical available solid mass at around 100s further indicates that the z-factor from the traditional fit is unsuitable for describing the observed dissolution process. When the estimate is refined based on the numerical calculation method described by Wang and Nelson, a z-factor of 2.5×10^{-2} mL/mg/min is found that is able to explain the observed profile with realistic available solid masses and an estimated disintegration time of 380s that slightly exceeds the observed disintegration time (Fig. 6b). Further substantiating the plausibility of the refined z-factor, the authors stated that in order to get a good match of their simulations with an observed plasma profile in a clinical study, they had to increase the traditionally fitted z-factor obtained from *in vitro* data by a factor of 4.[5] However, they provided no experimental basis to justify the factor applied. It was simply stated that they assumed the observed profile to be heavily influenced by disintegration.[5] In fact, a justification for the use of an increased z-factor could have been made on the basis of the refined z-factor estimate, as this value is approximately 2.5 fold higher compared to the value of the traditional fit. However, an increase by a factor of 4 as reported by the Ding et al.[5] cannot be supported, as the observed disintegration time would no longer match the theoretical available mass range (with estimated complete disintegration at ~400–500s, Fig. 6c).

Case Example II: Experimental Data From Li et al.[7]

Similar to the previous case example, experimental dissolution data taken from the supplementary material of a recent publication by Li et al.[7] was analyzed in regards to the validity of the z-factors they used to describe the observed dissolution process of commercially available piroxicam tablet formulations containing 10 mg of API. Piroxicam is a weakly acidic drug with a reported solubility of 93.8 μg/mL at pH 1.0. For the case example shown here (Fig. 7a), Li et al. performed the dissolution experiments with 10 mg piroxicam tablets in a USP II apparatus with 900 mL of 0.1 M HCl at 75 RPM (Fig. S17 in the supplementary data to their original article[7]). When the complete dose of 10 mg is assumed to dissolve completely in this medium, the resulting concentration (11.1 μg/mL) is much lower than the equilibrium solubility (93.8 μg/mL), again allowing sink conditions to be assumed for this case example.

As is clearly visible in Fig. 7a, a z-factor of 0.526 mL/mg/min as reported by Li et al.[7] is inappropriate for describing the dissolution process in this medium, as available masses as high as 200% of the dose used in the experiment would be required at certain time points. Again, for refining the z-factor estimate, the observed disintegration time needs to be taken into account. They reported the disintegration time obtained from compendial disintegration testing in distilled water at 37 °C to be in the range of 360–450s. This may not translate to an identical disintegration time in the USP II apparatus, especially when using a different medium, but can still serve as an estimate of the disintegration time. Further, as depicted in Fig. 7a, the dissolution process is incomplete as indicated by a stable plateau at around 75% mass dissolved. This implies that by the end of the dissolution experiment, ~25% of the drug mass has still not been made available for dissolution. As the z-factor is fitted assuming the full dose of the API to be available for dissolution, this is an additional source of error that eventually leads to an underestimation of the particle dissolution rate. Considering those aspects, a more realistic z-factor with a value five times greater than the one they reported is obtained when adopting the Nelson and Wang method[9] (Fig. 7b). This value is in agreement with the observed disintegration time and takes into account the plateau of the dissolution profile (i.e. the maximum available mass is assumed to be the highest observed cumulative mass released instead of the complete dose).

Many more examples can be found in the supplementary material of the study performed by Li et al.[7] where z-factors are underestimated to a large extent and are thus very likely to confound the results obtained in *in silico* simulations of plasma profiles. Similar to the case example discussed previously, it would have been very beneficial for their data evaluation to verify or refine the obtained z-factors with the aforementioned approaches.

Case Example III: Influence of Coning on the z-Factor Estimate

Besides the disintegration process of the dosage form, coning may also limit the solid mass available for dissolution. Fig. 8 depicts two case examples where, for some dosage forms, the amount of mass being available for dissolution was reduced due to coning. Fig. 8a shows the mean dissolution profiles of tablets containing amoxicillin tested in different vessel types. While all other test

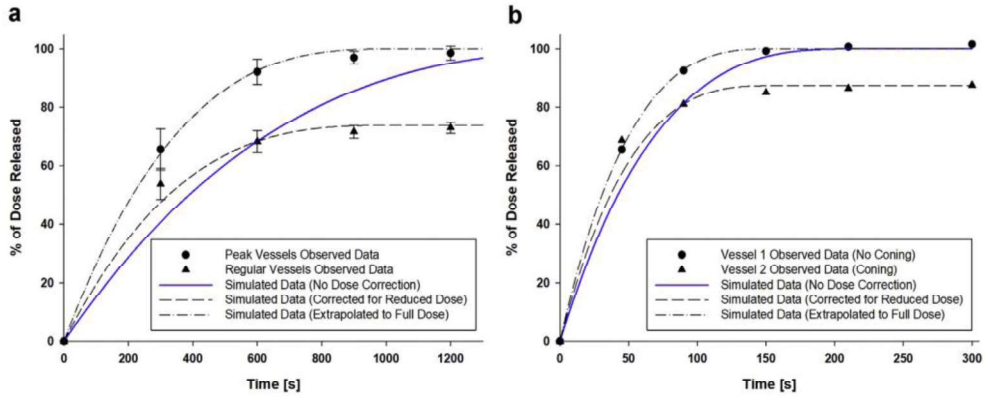

Fig. 8. Comparison of simulated dissolution profiles using traditionally fitted z-factors in dependence of the dose considered for calculation. (a) Release of 6 tablets containing 750 mg amoxicillin in peak vessels vs. regular USP II vessels. (b) Release of tablets containing 200 mg doxycycline monohydrate in two regular vessels with and without observed coning. Error bars depict the standard deviation in Fig. 8A.

conditions were maintained similar, it can clearly be seen that the occurrence of coning observed with regular vessels is avoided for the specific dosage form when peak vessels were used. As a second case example, Fig. 8b depicts the release profiles of two doxycycline monohydrate tablets under identical testing conditions for both tablets, where coning was observed in one vessel but did not occur for the other vessel.

When a traditional z-factor is fitted using the experimental data sets that show coning while wrongfully assuming the complete dose of the API for the calculation, z-factors are obtained that do not accurately describe the dissolution process of the dosage forms, neither in the absence nor the presence of coning (Fig. 8, solid blue lines). However, when the dose used for the z-factor fit is adjusted to be the maximum cumulative dose released for the cases where coning occurs, z-factors are obtained that, when applied to the respective dose, provide a good representation of the dissolution process with and without the occurrence of coning (Fig. 8, dash-dotted and dashed lines). For the case examples discussed here, it was therefore possible to extract z-factors that could accurately describe the particle dissolution process even when based on data sets that were confounded with coning.

Thus, when coning is observed during the dissolution experiment and the dissolution profiles show a significant plateau that indicates incomplete dissolution, it may be advisable to adjust the dose for the calculation of a *z*-factor to the value of the plateau in order to not underestimate the dissolution rate, provided that solubility is not the limiting factor for incomplete dissolution and when disintegration is either very rapid or negligible.

Scope of Application for the Refined z-Factor Estimate

As discussed in the theory section, the available mass range approach can be applied assuming both sink and non-sink conditions. In case of non-sink conditions, Eq. (8) has to be modified to include $[C_s - C(t)]$ instead of just C_s. In contrast, calculation of a theoretical disintegration profile using the method of Nelson and Wang[9] strictly requires sink conditions to apply. In the case of non-sink conditions, the life-span of individual particles would change over the dissolution time course, as the dissolution rate decreases with increasing drug concentration in the bulk medium. Thus, the calculation method for a theoretical disintegration profile would need to be expanded in order to include the dependence of the particle life-span on the total drug concentration in the media. For such cases, Horkovics-Kovats et al. have described an equation that takes into account non-sink conditions that can be applied to continuously measured dissolution data.[10] Using this equation, a theoretical disintegration profile analogous to the Nelson and Wang approach can be calculated numerically (with sufficiently small time steps for each iteration) for drug products where non-sink conditions have to be assumed.

The approach described here for separating the *in vitro* performance of a dosage form into particle dissolution and disintegration is most appropriate when the rates of these two processes are comparable (e.g. Dissolution Time/Disintegration Time = 0.5 to 1.5, Fig. 5). When disintegration is the rate-limiting step (e.g. for highly soluble drugs with a small particle size), both the traditional z-factor model and the refined approach become less accurate (Fig. 5). The slower disintegration is compared to the particle dissolution rate, the more it controls the observed dissolution profile of the dosage form, culminating an extreme case in which the observed dissolution profile matches the dosage form disintegration profile. Compared to the many mechanistic models that can describe particle dissolution, the process of disintegration is less well understood and appears to depend on a variety of underlying factors specific to the drug product (manufacturing parameters, choice and quantity of excipients and their interaction with the API and the medium etc.),[11] and is thus usually described empirically. If disintegration is rate-limiting to the dissolution of a drug product, experimental setups that reflect various physiological aspects relevant to dosage form disintegration, e.g. medium composition, pH, volume and temperature, hydrodynamics or the occurrence of pressure events should be used in order obtain biorelevant disintegration-dissolution profiles. Utilization of the GastroDuo apparatus for simulation of a variety of physiological scenarios is one example of an approach that has been shown to more accurately predict the *in vivo* disintegration and dissolution of certain dosage forms when compared to compendial apparatus.[12,13]

Another important aspect for the interpretation of dissolution results is the influence of manufacturing parameters on the resulting dissolution behavior of the dosage form. The granulation process, for example, can alter the dissolution properties of the API. On the one hand, granule size is by its nature coarser than that of API particles within the granule, thus reducing the surface area to volume ratio. On the other hand, granulation is often performed

with hydrophilic excipients, which may lead to faster dissolution, especially of poorly soluble APIs. Due to this ambiguity, difficulties in the description of granule dissolution with models that rely heavily on measured particle size distributions (such as the Johnson model[14]) may arise. While knowing the particle size is not a requirement for application of the traditional z-factor model (or, in case of substantial disintegration effects, the refined model), these models are best able to describe the effective dissolution rate of the API from granules when the particle size distribution is monomodal and very narrow. In cases where disintegration is the predominant aspect controlling the drug product's dissolution, or when broad and/or multimodal particle size distributions are present, utilization of the z-factor model is no longer reliable and other approaches are to be preferred. Examples are more sophisticated theoretical approaches that account for the expected mechanistic process of disintegration and multimodal particle size distributions,[15] or the use of *in situ* particle monitoring technology via focused beam reflectance measurement (FBRM) in order to assess the change in number and size of suspended particles during the dissolution process.[16,17] The latter can also be used to more precisely define the time until complete disintegration of a dosage form is reached, as this would coincide with the time where the maximum number of suspended particles are registered. Compared to the rather approximate disintegration recorded from visual observation, FBRM can provide a more reliable disintegration time for utilization in the refined z-factor model approach.[16]

Further limiting its applicability, the Nelson and Wang approach used here to find a z-factor for which the observed disintegration time matches with a calculated theoretical profile is viable only when strict sink conditions can be assumed, which is the case for very highly soluble drugs (e.g. Case example I) or for drugs with both a low solubility and a low dose, when the dissolution media volume is large enough to allow assumption of sink conditions (e.g. Case example II). Both approaches presented in this manuscript (the calculation of the available mass range and the numerical calculation of a theoretical disintegration profile) require either a continuously measured dissolution profile or parameterization of a non-continuous dissolution profile characterized by distinct time points that can be empirically described with a steady function. In the latter case, an additional error source is introduced as data points are interpolated. Furthermore, the choice of a mathematical function describing the observed dissolution data already defines the resulting shape of the profile, which can cause deviations from the 'real' disintegration profile. As an example, this can be seen in Fig. S3B, where the calculated disintegration profile approaches the plateau parabolically in contrast to the original disintegration profile which increases almost linearly and bends sharply when reaching the plateau. Despite the visible deviation, the obtained particle dissolution rate and the disintegration profile still result in an adequate description of the dissolution process, as minor differences are unlikely to have a meaningful impact on the simulation of the *in vivo* dissolution process, especially when occurring near the plateau of the observed profile.

In summary, the approaches to verify or refine a z-factor estimate presented here are reasonably applicable when sink conditions can be assumed. They offer a quick visual assessment of the suitability of a fitted z-factor to describe the dissolution process without the need for elaborate calculations and can be used to parameterize an observed dissolution profile for subsequent use in *in silico* simulations. While interpolating missing data points with an empirical function such as a Weibull distribution function undeniably constitutes a source of error that has to be critically reviewed, the case examples discussed here clearly show the superiority of the procedure compared to fitting a single dissolution factor from a complete dissolution profile that is confounded with disintegration and/or coning. In any case, when using default fitting approaches for a z-factor estimate, e.g. accessible in the commercial software GastroPlus™, the suitability of the resulting dissolution factor should be critically assessed in order to verify its subsequent use in modeling and simulation.

Conclusion

Based on the theoretical examples, the traditional z-factor fit approach is viable only when disintegration time is negligible compared to the expected dissolution time and no coning occurs. When disintegration is the rate limiting step for dissolution or coning occurs, the traditional z-value fit approach greatly underpredicts the particle dissolution rate. Furthermore, a forced fit through origin utilizing the complete dissolution profile data, is not advisable when considerable disintegration is observed, as this has led to the largest underestimation in the theoretical case examples. To refine the z-factor estimate, the observed disintegration time can be taken into account. However, this requires careful visual inspection of the vessels during the dissolution process. Careful selection of the suitable dissolution time points under consideration of the disintegration time resulted in a lower prediction error when particle dissolution time and dosage form disintegration time were in similar order of magnitude. When longer disintegration times are expected or observed, it is advisable to compare the traditionally estimated *z*-factor with the refined z-factor range obtained from the available mass range approach. Additionally, when sink conditions can be assumed, a more reliable z-factor can be estimated via numerical calculation of a theoretical disintegration profile that can be matched with the observed disintegration time.

Many examples of z-factors fitted to experimental data that are clearly confounded with disintegration and/or coning were identified in the literature, demonstrating the importance of considering the influence of such phenomena on the z-factor determination. The available mass range approach presented in this manuscript, along with the numerical approach by Nelson and Wang,[9] are suitable methods for verifying the suitability of *z*-factors obtained via the traditional fitting approach. For the interpretation of dissolution test results and a thoughtful assessment of a *z*-factor estimate, emphasis should be placed on visual inspection of the dissolution and disintegration process in order to obtain an estimate of the *in vitro* disintegration time which can be used to refine the estimate of a *z*-factor, eventually leading to a more adequate parameterization of the dissolution process for later use in *in silico* models.

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Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.xphs.2020.05.019.

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Evaluation of differences in dosage form performance of generics using BCS-based biowaiver specifications and biopharmaceutical modelling – case examples amoxicillin and doxycycline

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Evaluation of Differences in Dosage Form Performance of Generics Using BCS-Based Biowaiver Specifications and Biopharmaceutical Modeling—Case Examples Amoxicillin and Doxycycline

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ABSTRACT

Drug products containing the antibiotics amoxicillin (500 mg as trihydrate) or doxycycline (200 mg as hyclate or monohydrate) with varying qualitative excipient composition were obtained from the German market and their biopharmaceutical properties were characterized in compendial quality control tests, dissolution tests run under BCS-based biowaiver conditions and dissolution tests using biorelevant media. Observed differences in disintegration time and dissolution rate were assessed according to BCS-based biowaiver dissolution specifications and in virtual bioequivalence trials using GastroPlus™. Great variation was observed in dosage form performance, and 2 out of 5 drug products for each active ingredient failed to demonstrate *in vitro* similarity using the BCS-based biowaiver specifications, with coning being identified as a key hindrance. Nonetheless, all drug products investigated were found to be equivalent in virtual trials, concordant with their market approval status, indicating that the current BCS-based biowaiver criteria are over-discriminating. To bridge the gap between *in vitro* and pharmacokinetic assessment of bioequivalence, modification of the experimental setup with the use of Peak Vessels™ and the validation of dissolution specifications with virtual bioequivalence trials appear to be promising approaches. However, neither approach is currently foreseen by the harmonized ICH M9 BCS-based biowaiver guidance.

Introduction

Since the Biopharmaceutics Classification System (BCS) was introduced in 1995 by Amidon et al.,[1] the concept of classifying active pharmaceutical ingredients (APIs) based on their biopharmaceutical properties such as solubility over the physiologically relevant pH range (1.2–6.8) and surrogate parameters for permeability (e.g., fraction absorbed) for regulatory risk assessment purposes has led to the possibility of waiving the need for demonstration of *in vivo* equivalence during approval process of certain generic solid oral dosage forms, a procedure that is thus referred to as the *BCS-based biowaiver*.

As the regulatory criteria for a BCS-based biowaiver varied in several ways among guidance documents in different jurisdictions,[2–4] the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) drafted a harmonized guidance on BCS-based biowaivers ("M9") and the final version is currently recommended for adoption to the regulatory bodies of ICH regions.[5] Although the idea of harmonizing the BCS-based biowaiver procedure was well received in general, there were still concerns raised regarding the suitability of particular aspects of the regulatory criteria, and it was demonstrated that several drug products, although eligible for a BCS-based biowaiver in theory, failed to comply with the strict criteria for dissolution performance testing. These aspects were summarized in a recent publication.[6]

Two APIs for which the inability of dosage forms to comply with the regulatory criteria has been reported in the literature are amoxicillin and doxycycline (Figure 1). Drug products containing these antibiotic agents are listed on the World Health Organization (WHO) List of Essential Medicines (EML)[7] and are regarded as eligible candidates for a BCS-based biowaiver, as a positive risk-benefit assessment of waiving bioequivalence (BE) demonstration *in vivo* is documented in biowaiver monographs for both APIs.[8,9] However, strict interpretation of the ICH M9 guidance limits the applicability of the BCS-based biowaiver for both drugs, as

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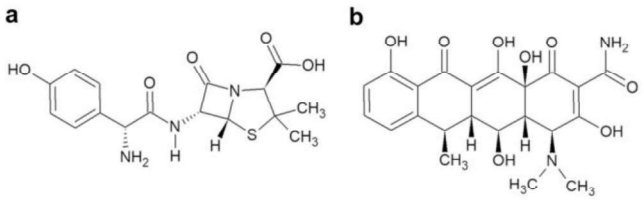

Figure 1. Molecular structures of (a) amoxicillin (b) doxycycline.

doxycycline monohydrate exhibits borderline BCS class I/II solubility, and amoxicillin shows instability in acidic media in addition to nonlinear pharmacokinetics and unfavorable BCS classification for doses greater than 750 mg (thus unnecessarily preventing the application of the BCS-based biowaiver to the 500 mg dose listed on the EML in some jurisdictions, whereas the BCS class of amoxicillin is established on the basis of a dose ≥750 mg). Further, as substantiated in several publications,[6,10–14] immediate release solid oral dosage forms containing these APIs are often unable to meet the criteria imposed by regulatory guidance documents. Thus, although a BCS-based biowaiver would be possible for drug formulations containing amoxicillin or doxycycline in theory, the BCS-based biowaiver approach in its current version is overdiscriminating, and fails to demonstrate the similarity of many of such drug formulations *in vitro*, even though *in vivo* studies have shown them to be bioequivalent.

The main reasons for the inability of some formulations to meet the criteria are likely attributed to the lower solubility and hence dissolution rate of these APIs at pH 6.8, but may also be associated with the occurrence of coning (i.e., a fraction of the solid drug being trapped in a zone with poor hydrodynamics), an *in vitro* artifact frequently observed in dissolution tests whose relevance for the *in vivo* situation is questionable.

In order to further investigate the reported problems, different tablet formulations containing amoxicillin (as the trihydrate) or doxycycline (as the monohydrate or hyclate) were obtained from the German market and tested according to the dissolution requirements of the BCS-based biowaiver. A thorough assessment of the biopharmaceutical properties and pharmaceutical quality control attributes of the formulations was performed with the aim of identifying differences in the dosage form performance *in vitro*. Further, the potential impact of such differences in dosage form performance on pharmacokinetic outcome parameters were studied in parameter sensitivity analyses and virtual bioequivalence trials using the modeling and simulation software GastroPlus™ (Simulations Plus Inc., Lancaster, PA).

The 2 APIs were chosen not only because of the documented cases where the products failed to meet the BCS-based biowaiver criteria, but also due to their individual biopharmaceutical and pharmacokinetic properties. Amoxicillin trihydrate in doses below 750 mg is considered highly soluble and highly permeable, resulting in a classification as a BCS I substance with eligibility for a BCS-based biowaiver. In higher doses, it has to be regarded as a BCS class II compound (doses of 750–1000 mg) or even a BCS class IV compound (doses exceeding 1000 mg) due to nonlinear absorption kinetics, which are most likely due to solubility limitations and saturation of the active transportation mechanisms via human peptide transporters (hPEPT), respectively.[9,15] Orally administered doses of 500 mg amoxicillin are almost completely and rapidly absorbed[16] with an absorption window for active transport via hPEPT in the small intestine[17–19] and thus show an early occurrence (t_{max} range of 1.0–2.5 h)[9] of the maximum observed blood plasma concentration, C_{max}. Amoxicillin is cleared with a short elimination half-life (1–1.5 h[16,20,21]) via renal filtration and secretion[22] as the major elimination pathway, with 43–80% of a dose being found in the urine as intact amoxicillin[21,23,24] and up to 25% as its inactive metabolite penicilloic acid.[25] For drugs such as amoxicillin that show early t_{max} and have a short pharmacokinetic half-life, Kortejärvi et al.[26] pointed out that C_{max}, one of the major bioequivalence outcome parameters, is particularly prone to be influenced by differences in the dissolution behavior of APIs.

In contrast to amoxicillin, doxycycline has a long pharmacokinetic elimination half-life (12–25 h[27]), high permeability (P_{app} [$\times 10^{-6}$ cm/s] = 17.5 ± 0.3 in a Caco-2 monolayer system[28]) and a late t_{max} of 1.5–3.5 h.[27] For such APIs, it was suggested that wider dissolution criteria could be acceptable for a BCS-based biowaiver, as minor differences in dissolution are unlikely to have an influence on pharmacokinetic outcome parameters for the assessment of bioequivalence.[26,29,30] Commercially available formulations of doxycycline contain the API in the form of either the monohydrate or the hyclate salt. The bioavailability of a 200 mg dose is high for both forms (mean fraction absorbed of 95%[27]). However, the two forms differ in their solubility: crystallizing the API as a hyclate salt results in an increased solubility and classification as a BCS I drug,[8] while doxycycline monohydrate slightly exceeds the critical dose/solubility limit of 250 mL and is thus to be treated as a borderline BCS I/II compound (see experimental solubility data in the results section).

The potential effect of differences in the biopharmaceutical behavior of amoxicillin and doxycycline drug formulations are of special interest in pharmaceutical practice, as drug formulations of both APIs are considered essential for an adequate public drug supply by the WHO[7] and are frequently prescribed in outpatient care: for example, immediate release solid oral dosage forms containing amoxicillin or doxycycline were both reported to be among the top 3 prescribed antibiotics on the German market in 2018[31] and show virtually complete generic market penetration in the statutory health insurance sector, as reflected in the relative market share of the top 3 generic manufacturers for the respective drug formulations, which was 100% for doxycycline and 98.8% for amoxicillin in 2017.[32]

In this study, 4 immediate release tablet formulations of amoxicillin and 5 formulations of doxycycline with authorization for the German market were identified that differ in their qualitative excipient composition in order to cover a broad range of dissolution performance and disintegration behavior. In order to investigate dissolution performance differences among drug products with similar qualitative excipient composition, an additional amoxicillin drug product was obtained that has a similar composition compared to one of the 4 obtained drug products, but is marketed by a different pharmaceutical company (*Amoxicillin AbZ* 500 mg *Filmtabletten* vs. *AmoxiHexal* 500 mg *Filmtabletten*).

Table 1
Qualitative Composition and Market Approval Details of the Generic Drug Products Obtained from the German Market

Generic Drug Product Name	Amoxicillin AL 500[C] (coated)	Amoxicillin Denk 500 mg Tabletten (withdrawn)	Amoxi-saar 500 mg	Amoxicillin AbZ 500 mg Filmtabletten (coated)	AmoxiHexal 500 mg Filmtabletten (coated)	Doxycyclin Stada 200 mg Tabs *Tabletten* (withdrawn)	Doxycyclin Heumann 200 mg Tabletten[C]	Doxycyclin 200 −1 A Pharma	Doxy-M-ratiopharm 200 mg Tabletten	Doxycyclin AL 200 T
First Market Authorization in Germany	April 18, 1984	December 17, 1992	February 01, 1994	February 04, 1985	August 13, 2010	April 04, 1990	January 15, 1990	May 14, 1992	April 04, 1990	July 25, 1990
API and Salt Form	Amoxicillin (as Trihydrate)					Doxycycline (as Monohydrate)				Doxycycline (as Hyclate)
Dose [mg]	500 (574 as Trihydrate)					200 (208 as Monohydrate)				200 (231 as Hyclate)
Average Tablet Weight [mg]	634[Exp]	684[GL]	958[Exp]	678[GL]	675[GL]	510[Exp]	381[Exp]	386[GL]	497[GL]	527[Exp]
Calculated Average Excipient Amount [mg]	60	110	384	104	101	302	173	178	289	296
Magnesium Stearate	Yes	Yes	Yes	Yes	Yes	–	Yes	Yes	Yes	Yes
Microcrystalline Cellulose	–	–	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Colloidal Silicon Dioxide	Yes	Yes	Yes	–	–	–	Yes	Yes	Yes	Yes
Povidone K25	Yes	–	–	Yes	Yes	–	–	Yes	–	–
Talc	Yes	–	–	Yes	Yes	–	–	–	Yes	–
Sodium Carboxymethyl Starch (Type A)	Yes	–	–	Yes	Yes	–	–	–	–	–
Titanium Dioxide	Yes	–	–	Yes	Yes	–	–	–	–	–
Corn Starch	Yes	–	–	–	–	Yes	–	–	–	Yes
Hydroxypropylmethylcellulose	–	–	–	Yes	Yes	Yes	–	–	–	–
Sodium Carboxymethyl Cellulose (Type A)	–	–	–	–	–	–	–	Yes	Yes	Yes
Macrogol 6000	Yes	Yes	–	–	–	–	–	–	–	–
Lactose Monohydrate	–	–	–	–	–	–	Yes	–	–	Yes
Hydrogenated Castor Oil	–	–	–	–	–	–	–	Yes	–	Yes
Eudragit E100	Yes	–	–	–	–	–	–	–	–	–
Sodium Croscarmellose	–	Yes	–	–	–	–	–	–	–	–
Hydroxypropylcellulose	–	–	Yes	–	–	–	–	–	–	–
Apricot Aroma	–	–	Yes	–	–	–	–	–	–	–
Saccharin	–	–	Yes	–	–	–	–	–	–	–
Vanillin	–	–	Yes	–	–	–	–	–	–	–
Crospovidone	–	–	–	–	–	–	Yes	–	–	–

C: Designated comparator product based on WHO selection criteria (first product introduced in the market or market leader when innovator is no longer available).[34]
GL: Information taken from the German drug product database "Gelbe Liste" (www.gelbe-liste.de).[35]
Exp: Experimentally determined average tablet weight of 3 tablets (relative standard deviation was <2% for all weights).

Based on regulations regarding the interchangeability of medicinal products as described in the framework contract according to article 129 of Volume V of the German Code of Social Law,[33] the various drug formulations of each API are considered similar and are thus substitutable in public healthcare.

Materials

Chemicals for Media Preparation and HPLC Mobile Phases

For preparation of the various media (see Table S1 for detailed compositions) for dissolution, disintegration, stability and solubility testing, the following chemicals of analytical grade were obtained from chemical suppliers: sodium dihydrogen phosphate dihydrate, potassium dihydrogen phosphate, di-sodium monohydrogen phosphate dodecahydrate and sodium dihydrogen phosphate monohydrate (Merck KGaA, Darmstadt, Germany); sodium chloride, phosphoric acid 85%, sodium acetate trihydrate, sodium hydroxide pellets, di-sodium edetate dihydrate (VWR International, Oud-Heverlee, Belgium); hydrochloric acid 37%, 1 M sodium hydroxide solution, 1 M hydrochloric acid solution, acetic acid 100%, ammonia solution 25% and acetonitrile (VWR International, Fontenay Sous Bois, France); tetrabutylammonium hydrogen sulfate (Thermo Fisher Scientific Inc., Bremen, Germany).

For fasted state simulating gastric fluid (FaSSGF) and the fasted state simulating intestinal fluid version 3 (FaSSIF-V3), ready-to-use powder preparations were supplied by Biorelevant.com Ltd. (London, UK).

All media were prepared using deionized water from in-house production of the Goethe University (Frankfurt am Main, Germany).

Commercial Drug Products

The following commercially available generic drug products (exclusively tablet formulations) with market authorization in Germany were obtained from the pharmaceutical wholesaler Phoenix Pharma SE (Mannheim, Germany):

Five generic drug products containing 500 mg amoxicillin (as trihydrate): Amoxicillin AL 500 (Lot: 72029, Aliud Pharma GmbH), Amoxicillin Denk 500 mg Tabletten (Lot: 19877, Denk Pharma GmbH & Co. KG), AmoxiHexal 500 mg Filmtabletten (Lot: FW1247, HEXAL AG), Amoxi-saar 500 mg (Lot: 2749300, MIP Pharma GmbH), Amoxicillin AbZ 500 mg Filmtabletten (Lot: GU8231, AbZ-Pharma GmbH).

Five generic drug products containing 200 mg doxycycline (as hyclate or monohydrate): Doxycyclin Al 200 T (Lot: 72032, Aliud Pharma GmbH), Doxycyclin 200–1 A Pharma (Lot: JH2145, 1 A Pharma GmbH), Doxycyclin Heumann 200 mg Tabletten (Lot: CDB9C001, Heumann Pharma GmbH & Co. Generica KG), Doxycyclin Stada 200 mg Tabs Tabletten (Lot: 42910, STADApharm GmbH), Doxy-M-ratiopharm 200 mg Tabletten (Lot: T03359D, ratiopharm GmbH).

Further details (first market authorization, average tablet weights and qualitative excipient composition) of the drug products tested in this study are compiled in Table 1.

As the originator drug products for doxycycline (Vibramycin, Pfizer GmbH) and amoxicillin (Amoxypen. Grünenthal GmbH) are no longer marketed in Germany, the respective generic drug products first introduced in the German market were chosen as the designated comparator drug products: Amoxicillin AL 500 and Doxycyclin Heumann 200 mg Tabletten.

Over the course of this study, 2 of the drug products (Amoxicillin Denk 500 mg Tabletten and Doxycyclin Stada 200 mg Tabs Tabletten) on the German market were discontinued (even though they still have market approvals).

Software

GastroPlus™ Version 9.7 (SimulationsPlus Inc.) was used for biopharmaceutical modeling and simulation of potential differences between the tested drug products. Excel 2016 (Microsoft Corporation, Redmond, CA) was used for data analysis (z-factor fit and theoretical disintegration profile calculation). SigmaPlot Version 11.0 (Systat Software Inc., San Jose, CA) was used for scientific graphing and data analysis.

Methods

In Vitro *Experiments*

Quantification of Dissolved API

For quantification of the amount of dissolved amoxicillin and doxycycline, compendial stability-indicating HPLC assay methods were adapted for both APIs. Adjustments were made in order to shorten the total run-time, avoid degradation of the APIs and take the preparation of samples from the different media into account. The adjusted methods were validated according to the ICH guideline on validation of analytical procedures.[36] Detailed information on the assay methods and validation parameters are given in Table S2.

During validation of the sample preparation and quantification, the stability of stock solutions of amoxicillin and doxycycline at physiologically relevant pH values (pH 1.2–6.8) and temperatures (25°C vs. 37°C) was determined. This was done to find the pH of optimal sample stability for quantification at room temperature, to determine first-order degradation constants at 37°C in various media for later use in the biopharmaceutical *in silico* model and to correct for the API loss due to degradation during the dissolution experiments. Details are provided in the supplementary material (Figure S1).

Solubility Determination

The solubility of amoxicillin trihydrate, doxycycline monohydrate and doxycycline hyclate after 4 h and 24 h incubation in various media covering the physiological pH range (1.2–6.8, Table S1) was determined using a small-scale shake-flask method. A moderate excess of substance based on observations of preliminary experiments was accurately weighed in Uniprep™ syringeless filter devices (GE Healthcare Ltd. Amersham, UK) consisting of 0.45 µm PTFE membrane filters inside a polypropylene housing. 3 mL of the respective media was then added and the samples were lightly shaken on an orbital shaker (Heidolph Polymax 1040, Heidolph Instruments GmbH & Co. KG, Germany) operating at a rotational speed of 40 RPM. The shaker was placed inside a laboratory incubator (Heraeus Model B 12, Heraeus Instruments, Germany) at a temperature of 37 °C ± 0.5 °C. The pH of the media was verified at the beginning of the experiment and was checked again after 4 h or 24 h, respectively. The dissolved amount of API was subsequently quantified via HPLC. All experiments were performed in triplicate.

Due to the high solubility of doxycycline hyclate (no solid residue in the Unipreps™ after 24 h) and the rapid degradation of amoxicillin in acidic media, the solubility of the 2 APIs below pH 2 was determined over 30 min instead of the 24 h solubility. A large excess of API was placed in Unipreps™ (n = 3) and 3 mL of preheated (37°C) simulated gastric fluid (SGF_{sp} pH 1.2 USP) was added. The samples were then vigorously shaken and placed on the orbital shaker inside the incubator. Every 10 min, the samples were

Table 2
Overview of Input Parameters and Variations From Default Options for the Biopharmaceutical Model of Amoxicillin and Doxycycline in GastroPlus™ V9.7

Parameter	Amoxicillin (Trihydrate)	Doxycycline (Monohydrate and Hyclate)
Physicochemical Parameters		
Molecular Weight [g/mol]	365.41[a]	444.44[a]
LogP	0.87[39]	−0.2[27]
pK_a Values	2.67/7.11/9.55[40]	3.02/7.97/9.15[41]
Reference Solubility [mg/mL]	3.06 (@pH 2.96)	Monohydrate: 0.693 (@pH 5.49) Hyclate: 1.53 (@pH 5.01)
pH vs. Solubility Profile	Fitted to experimental solubility data (Fig. S2)	
Diffusion Coefficient [$cm^2/s \times 10^5$]	0.691[a]	0.622[a]
Mean Precipitation Time [s]	1,000,000 (highest possible value as no precipitation was observed experimentally [Fig. S3])	
Dosage Form Parameters		
Dissolution Model	z-factor Dissolution (Takano et al.[42])	
Dosage Form Setting (For Tablet Formulations)	CRU: Gastric Release	
Disintegration Input	Parameters from fitted Weibull function	
Absorption Model Parameters		
Absorption Model	ACAT − Human Fasted State	
ASF Model	Opt logD Model SA/V 6.1	
Fluid Model	Dynamic Fluid Model/*Human − Dyn Vol 100% Mudie − Fasted*	
Effective Permeability [$cm/s \times 10^4$]	0.34[43]	1.68[b]
Active Transporters[b]	hPEPT K_m: 6.82 mM[b] V_{max}: 0.142 µmol/s[b]	N/A
Stomach Transit Time [h]/Gastric Emptying Half-Life [h]	0.284/0.2[29,44]	0.284/0.2[29,44]
Median Gastric Retention Time [h]	0.5[45]	0.5[45]
Median Fasted Gastric pH	2.7[45]	2.7[45]
%Residual Volume in Small Intestine (SI)/Colon	7.5 (SI)/2 (Colon)[46,47]	7.5 (SI)/2 (Colon)[46,47]
Pharmacokinetic Model Parameters		
Pharmacokinetic Model	Three-Compartmental Model (Details in Figs. S5 and S11)	
Fraction Unbound in Plasma	0.83[48]	0.18[49]
Blood/Plasma Concentration Ratio	0.93[a]	0.86[50] (from *in vitro* study using cattle blood)
Enterohepatic Circulation Settings[b]	N/A	Biliary Clearance Fraction: 0.733[b] Gallbladder Diversion Fraction: 0.75[b]

[a] Calculated/predicted From molecular structure with ADMET Predictor 9.5.
[b] Fitted with plasma profiles form the literature as explained in the methods section.

visually inspected for a solid residue inside the Unipreps™ and more API was added if no residue was observed. In either case, the samples were again shaken manually. After 30 min, both sets of samples showed a solid residue. They were filtered and the API concentration was quantified via HPLC.

Tablet Hardness Testing

Hardness of the different tablets was tested according to the procedure described in the European Pharmacopoeia (Ph. Eur. 9.7: *2.9.8 resistance to crushing of tablets*[37]) using an Erweka TBH30 MD hardness tester (Erweka GmbH, Langen, Germany). Three tablets of each brand were tested. In cases where the tablets had an oblong shape (Amoxi-saar, Amoxicillin AbZ, AmoxiHexal, Amoxicillin Denk), the hardness of 3 tablets was tested perpendicular to both the longer and the shorter dimension of the tablet. The force [N] needed to break the tablets was noted.

Disintegration Testing

Disintegration of the generic drug products was tested with the instrumental setup described in the European Pharmacopoeia (Ph. Eur. 9.7: *2.9.1 disintegration of tablets and capsules*[38]) for tablets of normal size (test A), using an Erweka ZT 3−4 disintegration tester (Erweka GmbH). Three tablets of each brand were tested in 4 different media. namely SGF_{sp} pH 1.2. phosphate buffer pH 2.7. acetate buffer pH 4.5 and SIF_{sp} pH 6.8 (Table S1). The tablets were placed into the cylindrical tubes with sieve bottoms. Afterwards, the device was submerged in the respective medium and was reciprocated vertically at 30 dips per minute. The time at which complete disintegration (i.e., a solid tablet core was no longer present) was achieved was determined visually and recorded.

Dissolution Testing

Dissolution of the generic drug products was tested in a USP II dissolution tester (Erweka DT 80; Erweka GmbH). As preliminary experiments showed a large influence of coning (as expected due to the high drug loads and excipient amounts depicted in Table 1) even at higher rotational speeds (75 rpm), Peak Vessels™ (Pharma Test Apparatebau AG, Hainburg, Germany) were used to reduce the impact of coning and to allow for lower and thus more discriminating paddle rotation speeds. The same media that were used in the disintegration experiment were also used here. Tablets were subjected to 500 mL of media (deaerated via vacuum filtration) at 37 °C ± 0.5 °C stirred at 50 RPM. When a release of ≥85% of the nominal API content was not achieved in ≤15 min, additional dissolution experiments were carried out with a rotational speed of 75 RPM. Samples were taken at time intervals recommended in the FDA guidance[3] (i.e., 5, 10, 15, 20, 30 min). However, shorter time intervals (e.g., 0.75, 1.5, 2.5, 3.5, 5, 7, 8.5, 10 min) were chosen when dissolution was observed to be very rapid, in order to accurately capture the early dissolution time points and the overall dissolution profile shape. During sampling, 5 mL of fluid was withdrawn via stainless steel cannulas equipped with 10 µm poroplast filters (Erweka GmbH) and filtered through pre-wetted 0.45 µm PTFE filters (Whatman™ Rezist, GE Healthcare Ltd.), returning the first 4 mL back into the dissolution vessel. The residual volume was appropriately diluted, using micropipettes (Eppendorf Research, Eppendorf AG, Hamburg, Germany) for doxycycline samples and positive displacement pipettes (Pos-D; Mettler-Toledo Rainin, LCC, Oakland, CA) for amoxicillin samples, to obtain a concentration in the range of the calibration curve and to achieve a sample pH that provided optimal stability for later quantification. Resulting

concentrations were mathematically corrected for the withdrawn sample volume, evaporation of media and API degradation (if applicable). For selected drug formulations (Amoxicillin Denk, Amoxicillin AbZ, Doxycyclin Heumann, Doxycyclin Stada), the release in 500 mL SIF_{sp} pH 6.8 was further compared to the release in 500 mL FaSSIF-V3 pH 6.7, stirred at 75 RPM. All experiments were conducted in triplicate.

Results of the dissolution testing were compared using the criteria for a BCS-based biowaiver for BCS class I APIs as described in the harmonized guidance (≥85% release in ≤15 min or ≥85% release in ≤30 min and dissolution profile comparison via f_2-test). Additionally, the time needed for complete disintegration of the tablets (examined visually) was noted for comparison with the compendial disintegration test.

Transfer Test

In order to investigate the possible occurrence of precipitation, transfer experiments were conducted using a USP II dissolution tester (Erweka DT 600, Erweka GmbH) with 3 mini vessels (Erweka GmbH: volume capacity: 400 mL) serving as the donor compartment and 3 regular vessels (Erweka GmbH; volume capacity: 1000 mL) serving as the acceptor compartment. Stock solutions of amoxicillin trihydrate (22.5 mg/mL amoxicillin) and doxycycline monohydrate (3.4 mg/mL doxycycline) in 250 mL FaSSGF pH 1.6 were used as medium in the donor compartment. 350 mL of FaSSIF-V3 pH 6.7 was used as the medium in the acceptor compartment. Both compartments were maintained at 37 °C ± 0.5 °C and stirred at 75 RPM. Media was transferred from the donor into the acceptor compartment using a peristaltic pump (Ismatec IP65; Cole-Parmer GmbH, Wertheim, Germany) with a programmed first-order transfer rate (half-life: 9 min). The pH in the acceptor compartment was frequently monitored and, when necessary, adjusted with defined volumes of 1 M sodium hydroxide solution in order to maintain a stable pH. Sampling at specified time points (1, 3, 5, 7, 9, 11, 15, 20, 25, 30, 35, 45, 60, 70, 80, 90, 120 min) from the acceptor compartment and quantification of the API content was performed analogous to the procedure described in the previous section.

In Silico *Modeling and Simulation*

GastroPlus™ Model Setup

In addition to the parameterization of the experimental data for use as input parameters in GastroPlus™, the scientific literature was searched for information on physicochemical and pharmacokinetic properties of the 2 APIs. An overview of the model setup is given in Table 2.

Once physicochemical and basic pharmacokinetic parameters were gathered, three-compartmental pharmacokinetic models were set up using data from clinical studies where doxycycline and amoxicillin were administered as intravenous solutions[51,52] (amoxicillin: Figs. S4-S6, doxycycline: Figs. S9-S11). As doxycycline undergoes enterohepatic recirculation, the compartmental pharmacokinetic model was established based on a study performed by Nguyen et al.,[51] in which the effect of oral coadministration of antacids on the plasma concentration of intravenous doxycycline was investigated in a crossover trial. First, a three-compartmental model was established based on the trial with coadministered antacids, in which the reabsorption of doxycycline due to entero-hepatic circulation was prevented (Figure S9). Afterwards, the *in silico* parameters used for modeling enterohepatic recirculation in GastroPlus™ were fitted using the data set of the clinical trial without coadministration of antacids (Figure S10).

After the basic compartmental pharmacokinetic models for i.v. administration were set up for both drugs, they were used for modeling the distribution and elimination subsequent to oral absorption of the respective drug. The absorption process from the gastrointestinal tract was simulated with the GastroPlus™ Advanced Compartmental and Transit (ACAT) model.[53] Changes to the default settings are indicated in Table 2. Simulating the human gut physiology, the fasted state dynamic fluid model (*Human – Dyn Vol 100% Mudie – Fasted*) was used to simulate fluid transition across the individual compartments as well as fluid secretion and absorption. Adopting the setup for residual fluid volumes used by Pepin et al.,[47] default values of 4.91% for the small intestine and 0.75% for the colon were adjusted to 7.5% and 2%, respectively, in order to match experimental values reported by Schiller et al.[46] and Mudie et al.[54] for intestinal fluid volumes in the fasted state. Stomach transit time was set to 0.284 h, corresponding to the mean gastric half-emptying time of 11.8 min after intake of 200 mL of water as reported by Oberle et al.[44]

In order to verify the literature values for human effective permeability in jejunal enterocytes (amoxicillin) or effective permeability values calculated from a correlation based on a CaCo-2 permeability assay reported in the literature[28] (doxycycline), studies administering the APIs as oral liquid dosage forms were used to match simulated and observed plasma profiles. The effective permeability P_{eff} for doxycycline was fitted to a value of 1.68×10^{-4} cm/s using the mean plasma profile for administration of an oral solution as reported in a study performed by Campistron et al.[55] (Figure S12). This value is in accordance with data from CaCo-2 studies performed by Saitoh et al.,[28] where an apparent CaCo-2 permeability of 17.5×10^{-6} cm/s was reported, which yields an effective permeability value of 2.13×10^{-4} cm/s when converted in GastroPlus™ with a calibration using effective permeability values summarized by Lennernäs et al.[56] and corresponding CaCo-2 permeabilities for the substances studied by Saitoh and coworkers.[28] As amoxicillin is actively transported via hPEPT in the small intestine, a corresponding transporter was added to the *in silico* model, and K_m and V_{max} values were fitted with the GastroPlus™ optimization module to 6.82 mM and 0.142 µmol/s, respectively, to match plasma profiles reported in the literature for orally administered liquid dosage forms of 250–1000 mg amoxicillin[57] (Figure S7).

As the last step, data from bioequivalence studies in which the drugs were administered as immediate release tablet formulation were used to verify the suitability the models[57] (amoxicillin: Figure S8, doxycycline: Figure S13).

Parameterization of Experimental In Vitro *Data for Input in the Biopharmaceutical Model*

Experimental solubility data of amoxicillin trihydrate, doxycycline monohydrate and doxycycline hyclate were fitted in GastroPlus™ V. 9.7 to obtain a continuous pH vs. solubility profile (Figure S2). The first-order degradation rate constants at different pH values determined for amoxicillin trihydrate (Figure S1) were used to simulate chemical degradation in the biopharmaceutical model. As no precipitation was observed in the transfer experiments (Figure S3), the precipitation time in GastroPlus™ was set to the maximum value (1,000,000s) for both APIs.

To investigate the maximum impact of differences in the dissolution behavior of the generic drug products, experimental data from dissolution tests using the slower rotational speed of 50 RPM were parameterized and used as input for the simulations. The experimentally obtained dissolution results were divided into 2 separate theoretical processes: dissolution of individual drug particles (z-factor[42] based) and, where applicable, disintegration of the dosage form. For drug products with very rapid disintegration

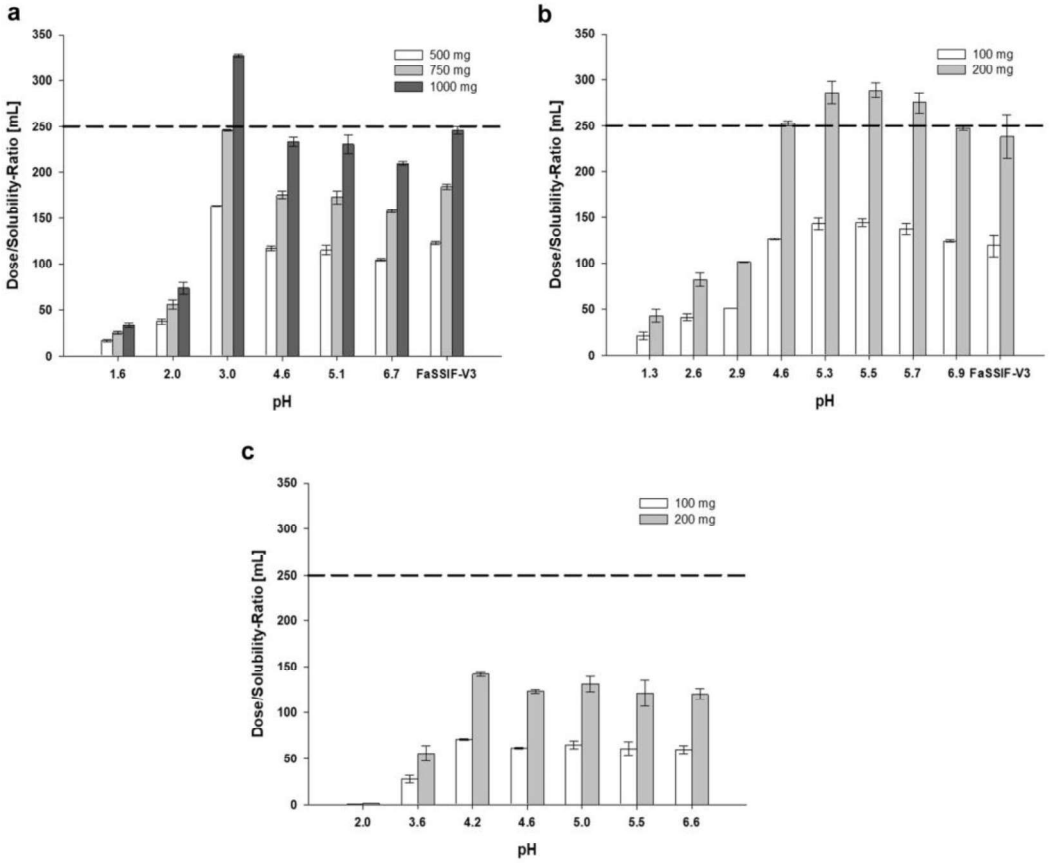

Figure 2. Experimentally determined dose/solubility-ratios (D/S) of (a) amoxicillin trihydrate (b) doxycycline monohydrate (c) doxycycline hyclate. Depicted pH values are the median pH values measured at the end of the experiment. Error bars indicate standard deviations (n = 3). Except for the amoxicillin D/S at pH 1.6 and the doxycycline hyclate solubility at pH 2.0 which are based on the 30 min solubility, as described in the method section, all shown D/S were calculated based on the solubility measured after 24 h.

(Amoxi-saar and doxycycline monohydrate products), linear disintegration with complete disintegration within 2 min was assumed. For the drug products that showed longer disintegration times, theoretical disintegration profiles were calculated based on a numerical approach described by Nelson and Wang[58] using the amount of dissolved mass calculated from a Weibull function fit of the dissolution profile. Assuming sink conditions, the theoretical mass of undissolved API available for dissolution was calculated for each time point using intervals of 1 s. The z-factor for each drug product was determined empirically as the lowest z-factor value that allowed for a realistic available mass profile (i.e., without having to exceed the total API dose or the occurrence of negative masses during the dissolution process). The calculated available mass profiles for the empirically determined z-factors were subsequently matched with the experimentally observed disintegration times via extrapolation of the ascending part of the available mass profile to obtain theoretical disintegration profiles. Detailed information on the dissolution parameterization of the drug products can be found in the supplementary material (Figs. S14-S25, Tables S3-S4).

In Silico Trials

In a parameter sensitivity analysis, the impact of changes in gastric emptying time (GET) or gastric pH on the pharmacokinetic parameter C_{max} was simulated for representative formulations of each drug product group. Gastric pH was varied between pH 1.2 and 4.5, and simulated gastric transit time was varied between 0 and 0.8 h, corresponding to a gastric emptying half-life of 0–33 min.

The drug products were further compared in virtual bioequivalence trials. The number of virtual subjects was chosen based on the average number of subjects enrolled in the *in vivo* bioequivalence studies that had been used for verification of the *in silico* model (12 for amoxicillin, 18 for doxycycline). Gastrointestinal fluid volumes, pH values and transit times of ACAT model compartments as well as Weibull parameters of the fitted disintegration profiles were selected as intra-subject variability parameters and thus varied among periods in the simulated crossover trials. Specifically, in order to closely match observed physiological gastric pH[45] and gastric emptying half-life,[44] the default variability value in GastroPlus™ had to be increased. The mean gastric pH of 2.7 was varied over a

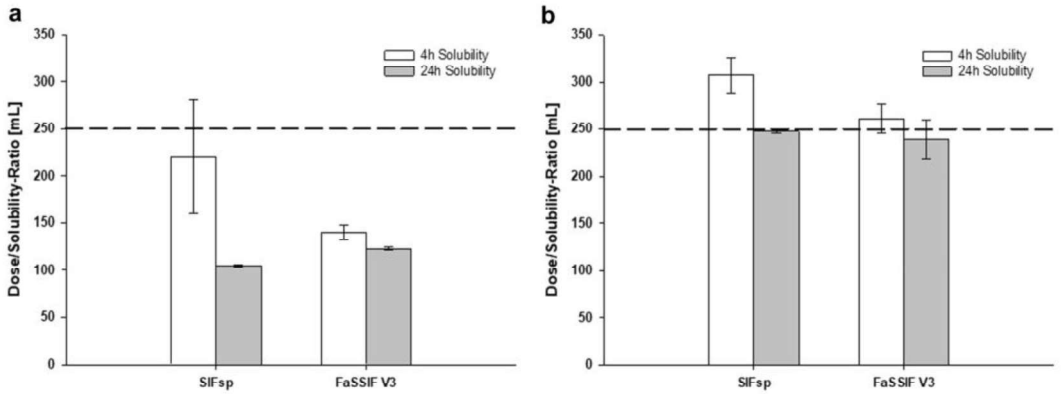

Figure 3. Comparison of dose/solubility ratios in the compendial medium SIFsp pH 6.8 and the biorelevant medium FaSSIF-V3 for (a) amoxicillin trihydrate (500 mg amoxicillin dose used for calculation) (b) doxycycline monohydrate (200 mg doxycycline dose used for calculation) after 4 h and 24 h of incubation. Error bars indicate standard deviations (n = 3).

range of 1.1–4.3 and the mean gastric emptying half-life of 11.8 min was varied over a range of 3–21 min. Other parameters were selected for inter-subject variability and the default variability of the GastroPlus™ population simulation setup was applied. Detailed information on the range of simulation parameters generated for the individual subjects in the virtual bioequivalence trials can be found in the supplementary material (Tables S6-S7).

Results and Discussion

In Vitro *Experiments*

Stability of Aqueous Solutions Over the Physiological pH Range

Results of the degradation experiments are depicted in Figure S1. While aqueous solutions of doxycycline did not show significant degradation over the physiological pH range (less than 10%[5]) and were stable at room temperature for ≥42 h when the pH was adjusted to 1.2, aqueous solutions of amoxicillin were prone to degradation with the degradation rate being dependent on media pH and temperature. The first order degradation rates (percent of drug amount degraded per hour) fitted to the degradation profiles obtained at 37°C were 14.16 ± 0.05 (pH 1.2), 2.51 ± 0.13 (pH 2.7), 1.6 ± 0.7 (pH 4.5), and 1.34 ± 0.16 (pH 6.8), respectively. Solutions of amoxicillin were found to be most stable at pH 6.0, with ≥98% of the initial amount still intact after 12 h at room temperature (25°C).

Solubility Over the Physiological pH Range

Experimental solubility values are depicted in Figure S2 and Table S5. Dose/solubility-ratios (D/S) calculated for different dosage form strengths are shown in Figure 2. In Figure 3, a direct comparison of solubility values measured after 4 h and 24 h in SIF_{sp} and FaSSIF-V3 is displayed.

The measured solubility values reported here are in accordance with the solubility values and the solubility classification reported in the biowaiver monographs for amoxicillin[9] and doxycycline.[8] As shown in Figure 2, doses of amoxicillin trihydrate ≤750 mg (based on pure amoxicillin) can be reliably classified as *highly soluble*, while higher doses exceed the critical D/S of 250 mL at pH 3.0 and have thus to be classified as *not highly soluble*. Regarding doxycycline, there is a clear difference in the solubility measured after 24 h for the 2 solid state forms examined: doxycycline hyclate is highly soluble over the physiological pH range, while the D/S for doxycycline monohydrate slightly surpasses the limit of 250 mL at pH values in the range of 4.5–6.8 for a 200 mg dose of doxycycline and is therefore either to be treated as a borderline highly soluble API or, imposing strict criteria, has to be treated as a compound that is *not highly soluble*. For both doxycycline monohydrate and amoxicillin trihydrate, there was no significant increase in the value of the 24 h solubility in the biorelevant medium FaSSIF-V3 (pH 6.7) compared to the compendial buffer solution SIF_{sp} (pH 6.8). However, it was observed that the solubility values in FaSSIF-V3 measured after 4 h were closer to the final solubility values measured after 24 h compared to experiments performed with SIF_{sp} (Figure 3). This implies that the equilibrium solubility is reached faster when using the biorelevant medium, reflecting a faster dissolution of the drug particles in FaSSIF-V3 even though the equilibrium solubility is comparable to SIF_{sp}.

Transfer Experiments

Results of the transfer experiment are shown in Figure S3. Neither doxycycline nor amoxicillin solutions showed precipitation when drug solutions in 250 mL FaSSGF (pH 1.6) were transferred into 350 mL of FaSSIF-V3 (pH 6.7), and the observed data closely match the theoretical drug concentrations calculated from the applied transfer rate under the assumption that no precipitation would occur. The final drug concentrations in the transfer experiments observed after 2 h were higher than the respective thermodynamic solubility values in FaSSIF-V3, indicating supersaturation for the concentration range used in the transfer experiment. The contribution of precipitation to the biopharmaceutical behavior of the drug products *in vivo* was therefore deemed negligible and was thus excluded in the *in silico* simulations (precipitation time was set to the highest possible value in GastroPlus™).

Tablet Disintegration and Hardness Testing

Median disintegration times of the tested dosage forms observed in compendial disintegration testing and dissolution testing using an agitation rate of 50 RPM in the USP II apparatus are shown in Figure 4. Tablet hardness depicted as median physical force needed to induce breaking of the dosage forms is depicted in Figure 5.

All tablets tested comply with the quality control requirements of the European Pharmacopoeia[59] regarding the specifications for

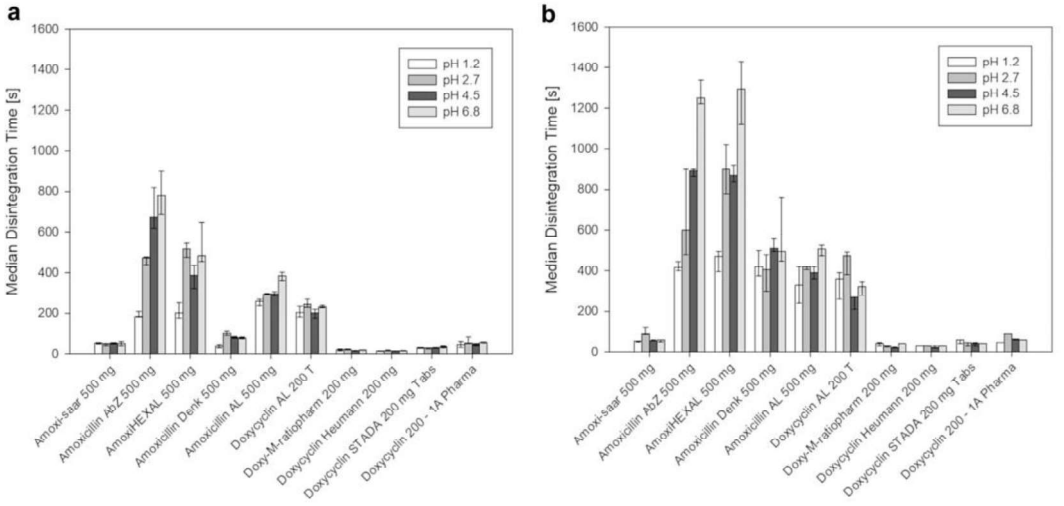

Figure 4. Median disintegration times of the dosage forms observed at different media pH during (a) compendial disintegration testing (b) dissolution testing performed at 50 RPM in the USP II apparatus. Error bars indicate the observed range (n = 3).

disintegration time of uncoated tablets (15 min) or tablets with film coating (30 min), respectively. However, large variability was observed in both the direct comparison of the disintegration times between the various drug products but also in the sensitivity of disintegration time to media pH for individual dosage forms containing amoxicillin trihydrate. While the uncoated tablets Amoxi-saar and Amoxicillin Denk completely disintegrated within 3 min, irrespective of the medium used in compendial disintegration testing, the other 3 drug products showed longer overall disintegration times and a clear dependence on media pH, with Amoxicillin AbZ exhibiting the broadest range of median disintegration times, from ~3 min in SGF_{sp} (pH 1.2) up to ~13 min in SIF_{sp} (pH 6.8). The longer disintegration time of Amoxicillin AbZ, AmoxiHexal and Amoxicillin AL may partly be explained by their film coating with hypromellose (AmoxiHexal and Amoxicillin AbZ) or Eudragit® E (Amoxicillin AL), respectively, which prevents immediate fluid penetration into the tablet surface and thus contact of the fluid with disintegrants in the tablet core. Another factor likely contributing to a longer disintegration time is the higher tablet hardness required to enable application of the film coating, which can additionally hinder rapid fluid penetration due to lower tablet porosity.

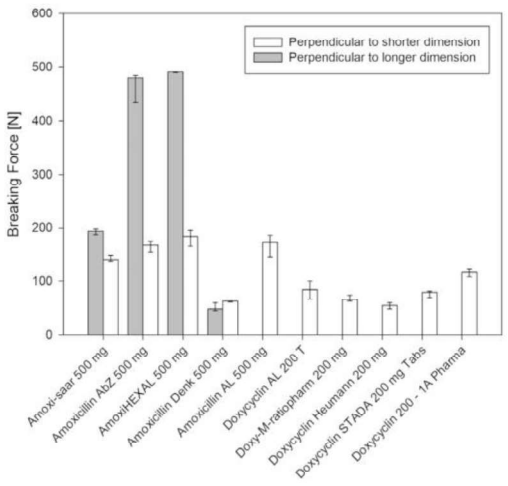

Figure 5. Median force [N] needed for breaking the tablets in compendial tablet hardness testing. Error bars indicate the observed range (n = 3).

When the tablets containing amoxicillin were subjected to dissolution experiments in a USP II apparatus operated at 50 RPM, all drug products (except *Amoxi-saar*) exhibited a considerable increase in disintegration times ranging from an average increase of 1.3-fold for Amoxicillin AL to 7-fold for Amoxicillin Denk. This may be explained by the different hydrodynamic conditions (rotational agitation in the USP II apparatus vs. uniform vertical displacement in the disintegration tester) as well as the absence of physical pressure on the tablets in the USP II apparatus. Pressure events were reported in experiments that subjected IntelliCap® devices into a compendial disintegration tester, while no such events were observed in a USP II apparatus.[60] These minor pressure events may have promoted faster disintegration of the tablets in a disintegration tester compared to the USP II apparatus, especially in the case of Amoxicillin Denk, for which low tablet hardness was observed.

Compared to the variability observed among drug products containing amoxicillin, disintegration of the doxycycline tablets was shown to be more robust towards changes in media pH and experimental setup. Complete disintegration of all drug products containing doxycycline monohydrate was achieved in less than 1.5 min for all experimentally imposed conditions and only minor differences were observed in the physical force needed to break the tablets during tablet hardness testing. However, compared to the drug products containing doxycycline monohydrate, it took longer for the drug product containing doxycycline hyclate (Doxycyclin AL) included in this study to completely disintegrate, especially

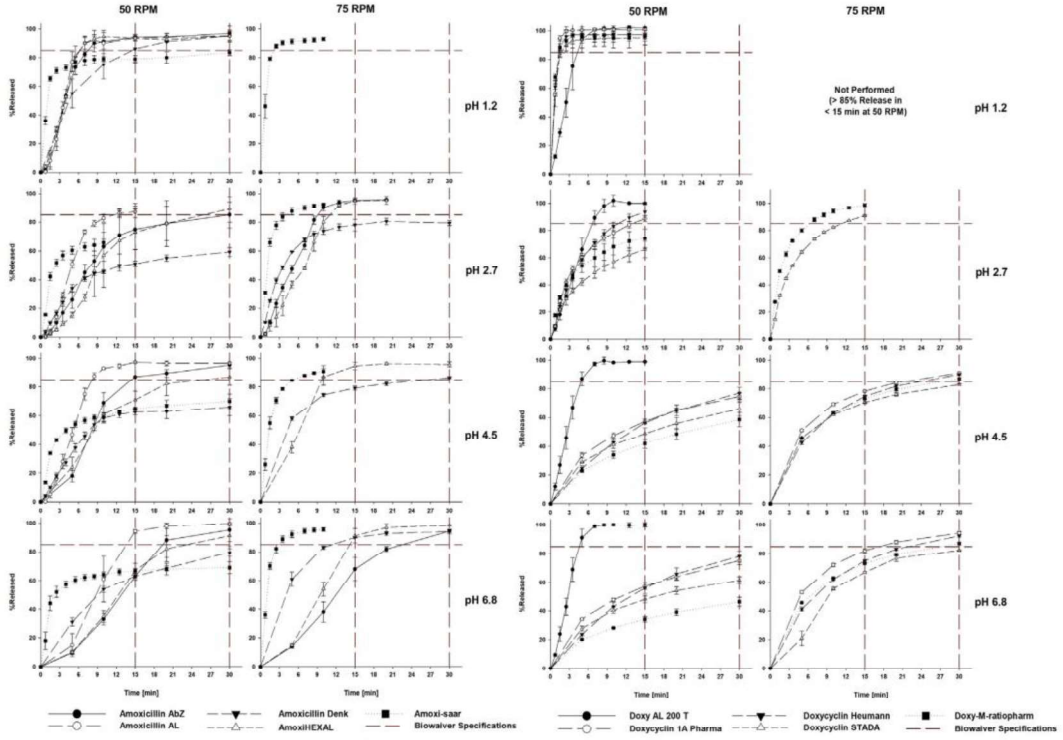

Figure 6. Release profiles observed in dissolution experiments using Peak Vessels™. When an amount ≥85% was released in ≤15 min at 50 RPM, the release at 75 RPM was not investigated. Left hand figures: dosage forms containing amoxicillin. Right hand figures: dosage forms containing doxycycline. Error bars indicate standard deviations (n = 3).

when subjected to the USP II apparatus operated at 50 RPM, where median disintegration times ranged from 4.5 to 8 min. Tablet hardness was in the range observed for the tablets containing doxycycline monohydrate.

Based on the results from the disintegration experiments, it is to be assumed that disintegration of the dosage form plays a major role in the dissolution behavior of drug products containing amoxicillin trihydrate (except for Amoxi-saar) and doxycycline hyclate. For Amoxi-saar and the drug products containing doxycycline monohydrate, disintegration was observed to be very rapid and the influence on the overall dissolution process is therefore of negligible importance compared to the dissolution rate of individual drug particles.

Dissolution Tests

A direct comparison of drug product dissolution profiles in the various media and agitation rates is presented in Figure 6. Table 3 summarizes the assessment of similarity of the dissolution behavior *in vitro* based on dissolution criteria given in the harmonized ICH guidance for the BCS-based biowaiver procedure.[5] In addition to the experiments performed with compendial media, Figure 7 shows a comparison of dissolution data obtained with the compendial buffer medium SIF_{sp} pH 6.8 and the biorelevant medium FaSSIF-V3.

Analogous to the variability observed in the disintegration experiments, the drug products containing amoxicillin trihydrate showed distinct differences in their dissolution behavior. The only drug product that was able to comply with the *very rapidly dissolving* (VRD) criterion for dissolution (≥85% release in ≤15 min) with an agitation rate of 50 RPM was Amoxicillin AL, the designated comparator product in this study. For Amoxi-saar and AmoxiHexal, the agitation rate had to be increased to 75 RPM in order for the products to meet the VRD criterion in every medium tested. Amoxicillin AbZ was VRD at pH 1.2 and 4.5 using 50 RPM as well as at pH 2.7 when using 75 RPM, but only complied with the *rapidly dissolving* (RD) criterion (≥85% release in ≤30 min) when tested at pH 6.8 at either 50 RPM and 75 RPM, preventing demonstration of similarity with the comparator product when compendial media are used. The only drug product not reaching either the VRD or RD criterion in every medium was Amoxicillin Denk, which just barely failed to achieve ≥85% release in ≤30 min when tested in a compendial phosphate buffer solution at pH 2.7, even when using 75 RPM.

For the doxycycline monohydrate products, decreasing dissolution rates were observed with increasing pH: while all drug products released >85% in 3 min at pH 1.2 using 50 RPM, and complied with the VRD criterion at pH 2.7 using 50 RPM (Doxycyclin Heumann and Doxycyclin 1 A Pharma) or 75 RPM (Doxycyclin Stada and Doxy-M-ratiopharm), none of the products showed very rapid dissolution at pH 4.5 and 6.8, and Doxycyclin Stada even failed to achieve ≥85% release in 30 min at 75 RPM, although only by a small margin (82.8 ± 0.7% and 82.3 ± 0.3% released after 30 min in acetate buffer pH 4.5 and SIF_{sp} pH 6.8, respectively). However, it still passed the f_2-test when tested

Table 3
BCS-Based Biowaiver Comparison of Analyzed Drug Products (Assuming Borderline BCS Class I for Doxycycline Monohydrate)

Comparator Product	Test Product	VRD or RD Criteria Fulfilled for Comparison?	f_2-Test Result	Demonstration of Equivalence Possible *In Vitro*?
Amoxicillin AL 500 mg	Amoxi-saar 500 mg	VRD (75 RPM/pH 1.2–6.8)	N/A	Yes
	AmoxiHexal 500 mg Filmtabletten	VRD (50 RPM/pH 1.2 and 75 RPM/pH 2.7–6.8)	N/A	Yes
	Amoxicillin AbZ 500 mg Filmtabletten	VRD (75 RPM/pH 1.2–4.5 & FaSSIF-V3) RD (50 RPM/pH 6.8) + f_2-Test	pH 6.8: 33.3	No (compendial media)
	Amoxicillin Denk 500 mg Tabletten	Test product fails to meet RD criteria (75 RPM/pH 2.7)	N/A	No
Doxycyclin Heumann 200 mg	Doxycyclin 200 1A Pharma	VRD (50 RPM/pH 1.2–2.7) RD (75 RPM/pH 4.5–6.8) + f_2-Test	pH 4.5: 64.0 pH 6.8: 52.6	Yes
	Doxy-M-ratiopharm 200 mg	VRD (50 RPM/pH 1.2 and 75 RPM/pH 2.7) RD (75 RPM/pH 4.5–6.8) + f_2-Test	pH 4.5: 59.1 pH 6.8: 71.4	Yes
	Doxycyclin Stada 200 mg Tabs Tabletten	Test product fails to meet RD criteria (75 RPM/pH 4.5–6.8)	pH 4.5: 65.2 pH 6.8: 46.9 FaSSIF-V3: 52.0	No (compendial media)
	Doxycyclin AL 200 T	VRD (50 RPM/pH 1.2–2.7) RD (75 RPM/pH 4.5–6.8) + f_2-test	N/A	No

VRD, very rapidly dissolving (≥85% release in ≤15min); RD, rapidly dissolving (≥85% release in ≤30 min); N/A, not applicable.

against the comparator product at pH 4.5. Doxycyclin Heumann, Doxy-M-ratiopharm and Doxycyclin 1 A Pharma all complied to the RD criterion when agitation rates of 75 RPM were used in dissolution media at pH 4.5 and 6.8 and further demonstrated similarity (f_2 >50) of the dissolution profiles (Table 3).

In accordance with the high solubility of doxycycline hyclate in the physiological pH range, Doxycyclin AL fulfilled the VRD criterion in all media tested at 50 RPM. However, as the designated doxycycline comparator drug product in this study (Doxycyclin Heumann) showed a considerably slower release in media at higher pH values (pH 4.5 and 6.8), demonstration of similarity of the respective dissolution profiles could not be achieved applying the regulatory criteria.

For each of the drug product groups, it was not possible to demonstrate *in vitro* similarity of dissolution profiles for 2 out of 4 test products assessed in compendial media applying the regulatory criteria for BCS class I drugs, even though Peak Vessels™ and agitation rates of 75 RPM were used to reduce the influence of coning. Even when applying the aforementioned conditions, pronounced coning was observed for Amoxicillin AbZ, AmoxiHexal, Amoxicillin Denk, Doxy-M-ratiopharm and Doxycyclin Stada and is thus to be regarded as a major aspect confounding the dissolution process, especially in the case of the doxycycline monohydrate dosage forms, which showed negligible disintegration times.

When FaSSIF-V3 was used as dissolution medium instead of SIF_{sp} (Figure 7), all drug products included in the comparison demonstrated faster release at the same agitation rate. Amoxicillin AbZ, which could only comply to the RD criterion in SIF_{sp}, was now very rapidly dissolving. Doxycyclin Stada, which barely failed to comply with the RD criterion in SIF_{sp} achieved ≥85% release in 30 min and demonstrated similarity of the release profile in comparison to the comparator product Doxycyclin Heumann in the same medium (f_2-test result: 52.0, indicating an average difference ≤10% between the profiles). Use of FaSSIF-V3 for simulating the environment of the small intestine therefore enabled demonstration of dissolution similarity with their respective

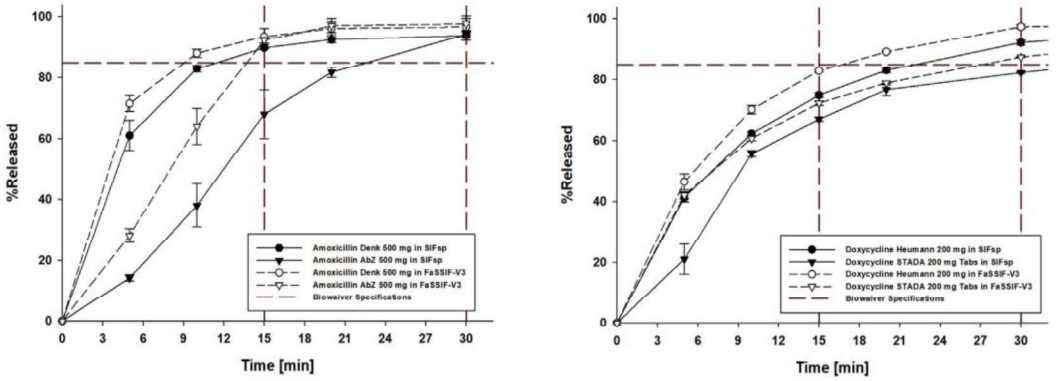

Figure 7. Comparison of dissolution profiles (USP II apparatus, Peak Vessels™, 500 mL medium, 75 RPM) in the compendial medium SIFsp pH 6.8 and the biorelevant medium FaSSIF-V3 for selected drug products containing amoxicillin trihydrate (left hand figure) or doxycycline monohydrate (right hand figure). Error bars indicate standard deviations (n = 3).

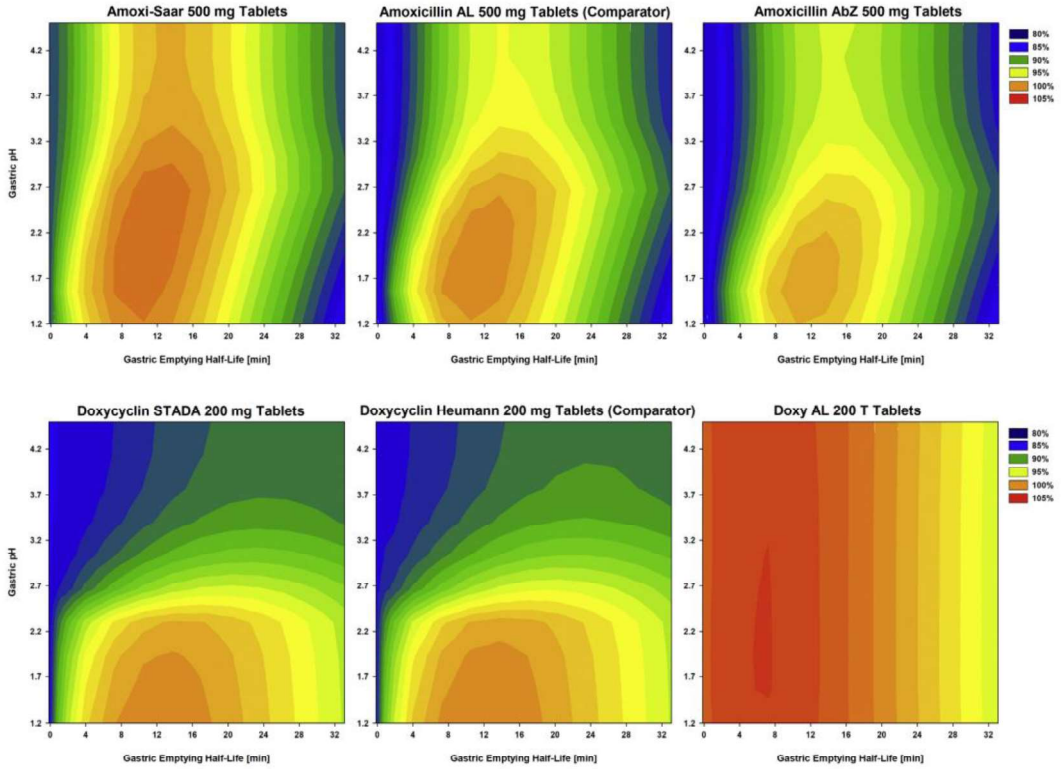

Figure 8. Parameter sensitivity analysis (PSA) of the impact of changes in gastric emptying rate and gastric pH on C_{max} for selected drug products containing amoxicillin (top figures) or doxycycline (bottom figures). Colored areas indicate C_{max} concentrations relative to the highest simulated C_{max} in the PSA of the comparator drug product for the respective API.

comparator drug product for 2 additional drug products tested (Amoxicillin AbZ and Doxycyclin Stada).

In Silico *Modeling and Simulation Results*

In order to assess the suitability of the regulatory guidance criteria for a BCS-based biowaiver and to investigate the theoretical influence of the observed differences in dissolution and disintegration behavior on pharmacokinetic outcome parameters (C_{max}, AUC) used for bioequivalence decisions, dissolution and disintegration processes were parameterized and virtual trials were simulated in GastroPlus™.

Among the drug products containing amoxicillin, Amoxi-saar showed the fasted dosage form disintegration and highest release rate, while Amoxicillin AbZ exhibited the longest disintegration time, slower particle dissolution rate and failed to demonstrate similarity of dissolution profiles with the comparator product at pH 6.8. Amoxicillin Denk was also not comparable to the comparator drug product. However, its inability to comply with the regulatory criteria was mainly attributed to strong coning, which was observed even when using higher agitation rates (75 RPM). Compared to Amoxicillin AbZ. it exhibited faster disintegration (Figure 4) as well as overall higher z-factor based dissolution rates (Table S3) and is therefore expected to release the API faster *in vivo*. Applying a bracketing approach, the parameterized dissolution and disintegration behavior of Amoxi-saar and Amoxicillin AbZ was subjected to virtual trials.

The bracketing approach was also applied to the drug products containing doxycycline. Doxycyclin AL, the drug product with the overall fastest release (and thus the only drug product for which similarity to the comparator product, Doxycyclin Heumann, could not be demonstrated under any of the applied *in vitro* conditions) was compared to the drug product with the slowest z-factor based release rates (Doxycyclin Stada, Table S3).

Parameter Sensitivity Analysis (PSA)

In addition to the drug products chosen for the bracketing approach, the designated comparator drug products (Doxycyclin Heumann, Amoxicillin AL) were included in the PSA.

Figure 8 shows the simulated influence of changes in gastric transit time and pH on the pharmacokinetic outcome parameter C_{max} for the selected drug products obtained from simulations performed with GastroPlus™ V.9.7.

For the drug products containing amoxicillin, differences in gastric emptying rate had a larger influence on simulated C_{max} compared to gastric pH. Highest C_{max} values were obtained when simulated gastric pH values were below 3 and simulated gastric half-emptying time was in the range of 5–20 min. Compared to the

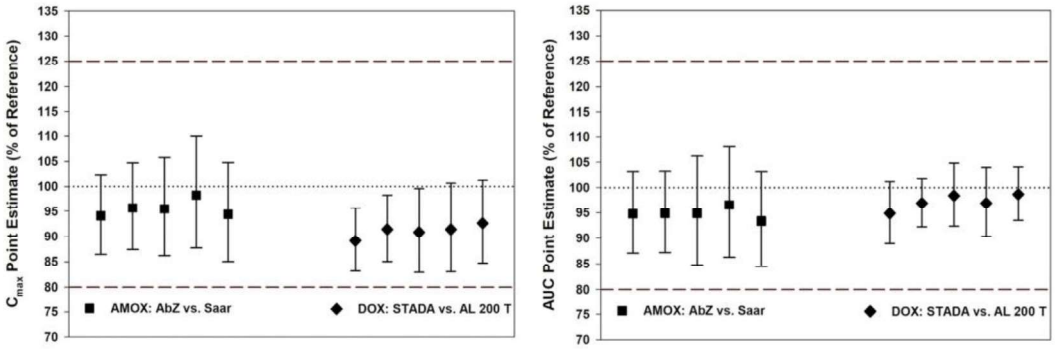

Figure 9. Point estimates for C_{max} (left hand figure) and AUC (right hand figure) from 5 virtual bioequivalence trials with n = 12 (amoxicillin) and n = 18 (doxycycline) virtual subjects, respectively, comparing the drug products with the slowest observed dissolution against the respective fastest ones (Amoxicillin AbZ vs. Amoxi-saar and Doxycyclin Stada vs. Doxycyclin AL). Error bars indicate the 90% confidence interval. Dashed lines depict bioequivalence specification limits (80.00−125.00%).

designated comparator product Amoxicillin AL, simulations with the fast dissolving and disintegrating drug product Amoxi-Saar yielded higher overall C_{max} values even when rapid gastric emptying was simulated, whereas they were slightly lower and more influenced by gastric emptying for Amoxicillin AbZ, as was expected based on its slower disintegration and lower estimated dissolution rate. Although C_{max} was observed to decrease with increasing dissolution and disintegration time, especially when gastric emptying was simulated to be rapid ($t_{1/2}$ <4 min) or slow ($t_{1/2}$ >24 min), respectively, the risk of the drug products being bioinequivalent *in vivo* can be regarded as low, as simulated C_{max} values were still above 90% of the highest value observed for the comparator product in the majority of the simulated scenarios. This is further substantiated by the fact that the range over which the highest C_{max} values were simulated covers the physiological range observed in clinical trials after administration of 200 mL of water to healthy adults, where the median gastric pH value was reported to be 2.7[45] and the grand mean of gastric half-emptying time was 11.8 ± 8.2 min.[44]

For the drug products containing doxycycline, the influence of the investigated parameters was heavily dependent on the respective salt form. While the simulated pharmacokinetics of Doxycyclin AL (containing the hyclate salt) were only minimally influenced by a change in gastric pH and exhibited only a slight decrease in C_{max} with lower gastric emptying rates, the monohydrate formulations were strongly influenced by changes in gastric pH. While C_{max} was still ≥95% of the highest observed value for pH values <2.0 and gastric half-emptying times >2 min, higher gastric pH values (>3.2), especially when combined with rapid emptying ($t_{1/2}$ <10 min), led to a decrease in C_{max} below 90%, in accordance with the lower solubility and slower dissolution rate of doxycycline monohydrate at higher pH. However, given that apart from these extreme scenarios, C_{max} was simulated to be above 90% of the highest value obtained in simulations with the comparator product, bioequivalence of the tested drug products is still expected in healthy subjects. However, differences in gastric pH could lead to a larger intra- and inter-subject variability.

Virtual Bioequivalence Trials

Figure 9 shows the results of the virtual bioequivalence trials performed adopting a bracketing approach comparing the drug product that showed the slowest release/disintegration time to the drug product with the fasted release/disintegration. As expected from the PSA, C_{max} and AUC point estimates (P.E.) and their respective 90% CIs were within the range of 80−125% in all of the simulated trials, thus complying with the specifications generally imposed in bioequivalence trials.

In contrast to the evaluation of the dissolution results *in vitro* using compendial media where similarity could not be demonstrated for 4 tested drug products, the conducted virtual bioequivalence trials support the assumption that, despite their observed differences in dissolution and disintegration performance, all drug products tested in this study would yield pharmacokinetic bioequivalence when tested in clinical trials, as the parameterized differences in dissolution rate and disintegration time did not lead to changes large enough to have an impact on outcome parameters in the virtual bioequivalence trials, even when the product with the fastest release was compared to the product with the slowest release. This finding is further supported by the fact that parameterization of the dissolution and disintegration behavior was based on the lowest agitation rate (50 RPM) used in the dissolution experiments with compendial media, where slowest release and longest disintegration times were observed. Physiological fluids are expected to facilitate particle dissolution in comparison to the buffers used in the BCS-based biowaiver, as when using biorelevant media faster dissolution times were observed compared to compendial media when release was tested under identical hydrodynamic conditions. An additional factor not taken into account for the *in vitro* experiments performed here are pressure events affecting the dosage form frequently observed in the course of gastric emptying *in vivo*,[60] which would likely facilitate disintegration of the tablets.

As the virtual bioequivalence trials all indicated comparable *in vivo* performance of the dosage forms whereas the BCS-based biowaiver specifications were over-discriminating in some cases, the possibility of extending the 'universal' regulatory dissolution specifications for a BCS-based biowaiver for doxycycline and amoxicillin was examined. Assuming dissolution under ideal sink conditions without disintegration *in vitro*, theoretical z-factor values were calculated for each API that would yield an amount dissolved of 85% of the dose in 15, 20, 30, and 40 min, respectively. The calculated z-factors were subsequently used as input parameters for dissolution in virtual bioequivalence trials and their influence on the outcome parameter C_{max} was compared to the *in silico* performance of the respective comparator drug product for amoxicillin and doxycycline. Results of the assessment are shown in Figure 10.

Compared to Amoxicillin AL, the comparator product, the 90% CI. of the C_{max} P.E. in virtual bioequivalence trials was within

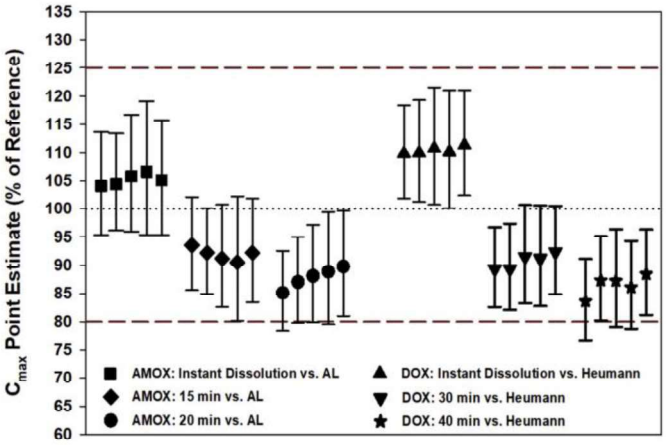

Figure 10. Point estimates for C_{max} of amoxicillin and doxycycline from virtual bioequivalence trials comparing the designated comparator drug product to different *in vitro* dissolution specifications. Error bars indicate the 90% confidence interval. Dashed lines depict bioequivalence specification limits (80.00–125.00%).

bioequivalence limits for z-factors corresponding to a theoretical *in vitro* release of 85% of the content in up to 15 min, but failed to remain above the lower bound of acceptance (80.00%) when the dissolution time was longer (Figure 10). This indicates that for demonstration of similarity *in vitro* with this comparator drug product, the BCS-based biowaiver VRD criterion is "safe" for amoxicillin but should not be extended further. While appropriate in theory, the application of the VRD criterion is problematic in practice, as it was not possible to demonstrate similarity to the comparator drug product for half of the generic amoxicillin products tested in this study, even when using Peak Vessels™ and 75 RPM.

For doxycycline, z-factor based dissolution rates that yielded ≥85% dissolved within 30 min resulted in the virtual BE trial complying with the acceptance limits. However, for longer dissolution times, the lower bound of the 90% confidence interval of C_{max} fell below the acceptance limit (80.00%) when compared to the comparator drug product, Doxycyclin Heumann (Figure 10). This confirms that both the VRD and the RD specifications are suitable for tablet formulations containing doxycycline for ensuring equal *in vivo* performance, without the need for f_2-testing. This relaxed RD dissolution specification would enable the demonstration of *in vitro* similarity between Doxycyclin AL 200 T and the comparator drug product Doxycyclin Heumann.

While simulated differences in C_{max} were greater and in several cases exceeded the regulatory limits for bioequivalence for longer dissolution times depicted in Figure 10 (>15 min for amoxicillin, >30 min for doxycycline), all corresponding 90% CI for AUC were within the bioequivalence criteria for the dissolution times investigated (data not shown). This is also frequently observed in real bioequivalence trials of oral drug products,[61–63] and shows that for successful demonstration of pharmacokinetic equivalence of drug products containing amoxicillin and doxycycline, C_{max} is the most critical parameter. This is expected due to the higher sensitivity of C_{max} to variability in gastric emptying time, dissolution performance and intestinal absorption rate. As expected with drug products containing *highly permeable* compounds, the total extent of API absorbed from the gastrointestinal tract, as reflected in the simulated AUC values, was not substantially influenced by variability in dissolution and disintegration times observed in this study.

Discussion

Despite being based on a "worst-case" approach considering the longest disintegration times and lowest dissolution rates observed *in vitro*, virtual bioequivalence trials using the parameterized dissolution and disintegration behavior of the dosage forms as input for the biopharmaceutical model was the only approach yielding similarity of all dosage forms of each API investigated in line with their market approvals. Comparison of the drug products using current BCS-based biowaiver criteria led to 2 drug products of each API being rejected when compared to the respective comparator drug product, even when using favorable dissolution conditions such as a rotational speed of 75 RPM and Peak Vessels™, and must therefore be deemed over-discriminating. Extension of the regulatory dissolution specifications based on a 'universal' dissolution time criterion could not be supported in virtual bioequivalence trials with amoxicillin, and only a minor room for extension for drug products containing doxycycline monohydrate was identified. These results suggest that differences in dosage form performance cannot be adequately assessed based solely on a single, universal dissolution time specification, but should rather be investigated with a biopharmaceutical model that accounts for the complex interplay between the gastrointestinal environment (pH, volumes, transit times) and dosage form behavior of the formulated API. This would enable a more comprehensive decision as to whether differences in dissolution rate or disintegration time are likely to be reflected in *in vivo* pharmacokinetics or not.

Surprisingly, regulatory BCS-based biowaiver dissolution specifications were found to be more suitable for doxycycline monohydrate, a borderline BCS class I/II compound, compared to amoxicillin trihydrate, which is a BCS class I compound at the dose applied in this study. This implies that in some cases, tying the dissolution specifications solely to a certain BCS class in a 'one size fits all' approach may be inappropriate, as this may lead to overly strict specifications or even exclusion from the biowaiver procedure on the one hand, but could also on the other hand lead to specifications being not strict enough. As the physicochemical, biopharmaceutical and pharmacokinetic properties are often well characterized for generic drug products, establishing BCS-based biowaiver dissolution specifications should preferably be centered

around these individual aspects in combination with the dosage form performance instead of simply relying on their BCS class.

When using *in silico* tools to support API-specific dissolution specifications for a BCS-based biowaiver, emphasis should lie on the suitability and validity of the model as well as appropriate parameterization of the *in vitro* dissolution data. As an example, Tsume and Amidon[29] have already proposed specifications for drug products containing amoxicillin in a previous study, stating that for amoxicillin, an amount of 85% drug released in 45 min would be adequate to ensure bioequivalence. However, their dissolution specifications were based on the release in the simulated gastrointestinal tract, which may not translate to the similar release rate in *in vitro* dissolution tests. For comparison, an amount of 85% of a 500 mg dose released in 10 min assuming sink conditions (which are usually present in *in vitro* dissolution tests of BCS class I compounds due to large media volumes) considering z-factor based dissolution would translate to an amount released of 85% in ~50 min in the GastroPlus™ model for amoxicillin used in the present study. This suggests that, in the case of amoxicillin, *in vitro* release times do not translate identically to the situation *in vivo*. Furthermore, the authors used a dose of 850 mg amoxicillin in their model setup and did not verify the suitability of their model with clinical data. Due to the nonlinear pharmacokinetics of amoxicillin that are attributed to saturable active transport and solubility limitations at higher doses, pharmacokinetics of a dose of 850 mg amoxicillin are less prone to changes in dissolution behavior compared to a dose of 500 mg, so that wider specifications could apply for higher doses. In the interest of establishing reliable and safe dissolution specifications, such specifications should always be based on the dosage form strength whose pharmacokinetics are most prone to changes in dissolution behavior. Therefore, the suitability of the dissolution specifications proposed for amoxicillin by Tsume and Amidon[29] are to be questioned and cannot be supported based on the simulation results obtained in this study, in which we have demonstrated that extension of the regulatory specifications is not supported for amoxicillin immediate release drug products.

While the results of the virtual bioequivalence trials generally confirm the suitability and safety of the BCS-based biowaiver criteria for amoxicillin and doxycycline, there is a limitation to the present study: as all drug products were obtained from the German market and are assumed to be interchangeable and thus bioequivalent, the outcome of this study must be regarded as a confirmation of the null hypothesis that observable differences in dosage form performance for these drug products have little or no meaningful impact *in vivo*. Further studies would need to be conducted with drug products that failed to demonstrate BE *in vivo*, in order to verify whether such observed differences can be simulated *in silico*, in order to establish meaningful dissolution specifications based on biopharmaceutical characterization. However, as reported in a retrospective assessment of bioequivalence trials,[64] bioinequivalence is rarely observed with BCS class I/III compounds, and *in vivo* inequivalent drug products containing APIs of these BCS classes are therefore difficult to obtain.

Conclusion

As exemplified by drug products containing amoxicillin and doxycycline, distinct differences in dosage form performance can be observed *in vitro* that do not necessarily translate to differences in the *in vivo* performance (as judged by the registration status of the products and *in silico* trials). BCS-based biowaiver dissolution criteria can therefore be regarded as safe but with a tendency to over-discriminate products containing APIs that are eligible for the procedure. This is particularly true for APIs that demonstrate a moderate absorption rate, long pharmacokinetic elimination half-life and late t_{max}, such as doxycycline.

For the release of the drug products investigated in this study, coning due to high drug or excipient amounts appeared to be a key hindrance to meeting BCS-based biowaiver requirements. Modifications to the experimental setup such as the use of Peak Vessels™ reduced the occurrence of coning and enabled lower rotational speeds to be used for comparison. Interestingly, drug formulations of both APIs showed slower dissolution rates at neutral pH values when tested in compendial buffers, as compared to when a biorelevant medium, FaSSIF-V3, was used. Modification of the experimental setup to include the use of Peak Vessels™ and biorelevant media in combination with virtual BE trials appears to be a promising approach to bridging the gap between *in vitro* and pharmacokinetic assessment of bioequivalence. However, none of these elements are currently taken into consideration by the harmonized M9 BCS-based biowaiver guidance.

Linking the observed dissolution behavior to the *in vivo* performance using the *in vitro/in silico* approach presented here enabled verification of the currently employed regulatory BCS-based biowaiver dissolution specifications. Further, the approach makes it possible to establish API-specific, safe-space dissolution criteria and an in-depth biopharmaceutical risk evaluation of the parameters that are crucial for bioequivalence, which can help to identify which drug products will be most robust towards variability in GI tract physiology. Applying this approach, it is possible to support a waiver of bioequivalence for individual APIs and their drug products that would otherwise fail the traditional BCS-based biowaiver criteria, thus reducing the regulatory burden in terms of requiring BE studies without adding to patient risk. Similar to the approach discussed for doxycycline monohydrate, where it was demonstrated that the regulatory biowaiver criteria are suitable even though the API could conservatively be classified as a BCS class II compound, the procedure could potentially be applied to other BCS class II drugs that share certain pharmacokinetic characteristics such as a moderate absorption rate and long elimination half-life or biopharmaceutical characteristics such as high solubility in the gastric environment without precipitation in the intestine.

Acknowledgements and Declaration of Interests

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Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.xphs.2020.04.011.

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A.2. Curriculum vitae

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Name:	Martin Andy Hofsäss
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Nationality:	German
Date of birth:	22.01.1991
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Academic and Professional Career

Jan 2016 – Apr 2020 **Goethe-University, Frankfurt am Main**

- PhD-Student/Research associate at the Department for Pharmaceutical Technology. Supervisor: Prof. Dr. Jennifer Dressman
  - Research project: *in vitro* release testing as an alternative to establishing bioequivalence of drug products *in vivo*
  - Teaching assignments: supervision of undergraduate practical courses in pharmaceutical technology, preparation and presentation of lectures and seminars on formulation development of sterile dosage forms.

Feb 2016 – Mar 2017 **Eschbach Apotheke, Frankfurt am Main**

- Employee (pharmacist) in part-time

Oct 2010 – Feb 2016 **Goethe-University, Frankfurt am Main**

- Pharmacy degree (final grade: 1.28)
- Pharmaceutical state examination (grades: 2.0, 1.0, 1.0)

2003 – 2010 **Felix-Klein-Gymnasium, Göttingen**

May 2010
- High school graduation / *Abitur* (final grade: 1.2)

Mar 2009
- Two-week stay at the Nanjing Foreign Language School, Nanjing, China, as part of a student exchange programme

2003 – 2007
- Classes with emphasis on mathematics and science

Internships

May 2015 – Oct 2015 **Radilo-Apotheke, Frankfurt am Main**

- Intern - Tasks included: sales, preparation of prescriptions, identity verification of chemicals

Nov 2014 – Apr 2015 **Goethe-Universität, Frankfurt am Main**

- Research assistant at the Department for Pharmaceutical Technology: supervision of preparatory courses in formulation development for undergraduate pharmacy students and experimental solubility determination of essential medicines for BCS classification

Aug 2011 – Sept 2011 **Marien-Apotheke, Göttingen**

- Intern – Tasks included: evaluation of the shelf-life and quality control of hospital drug inventories, assisting with the manufacture of sterile dosage forms

Mar 2011 – Apr 2011 **Gauss-Apotheke, Göttingen**

- Intern – Tasks included: incoming goods inspection, identity verification of chemicals

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Prof. Dr. Henning Blume, Prof. Dr. Hans Crauel, Prof. Dr. Theo Dingermann, Prof. Dr. Jennifer Dressman, Prof. Dr. Gunter Eckert, Prof. Dr. Eberhard Ehlers, Prof. Dr. Robert Fürst, Prof. Dr. Axel Helmstädter, Prof. Dr. Michael Karas, Prof. Dr. Jochen Klein, Prof. Dr. Jörg Kreuter, Prof. Dr. Hanns-Christian Mahler, Prof. Dr. Rolf Marschalek, Prof. Dr. Walter E. Müller, Prof. Dr. Eugen Proschak, Prof. Dr. Manfred Schubert-Zsilavecz, Prof. Dr. Holger Stark, Prof. Dr. Dieter Steinhilber, Prof. Dr. Mona Tawab, Prof. Dr. Markus Veit.

A.4. Statutory declaration (Eidesstattliche Erklärung)

ERKLÄRUNG

Ich erkläre hiermit, dass ich mich bisher keiner Doktorprüfung im Mathematisch-Naturwissenschaftlichen Bereich unterzogen habe.

Frankfurt am Main, den 07.03.2021 .....
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Versicherung

Ich erkläre hiermit, dass ich die vorgelegte Dissertation über

In vitro Release Testing as an Alternative to Establishing Bioequivalence of Drug Products in vivo

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Frankfurt am Main, den 07.03.2021 .....
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