Optimization of solubility studies performed in the context of BCS biowaiver monographs

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1. Introduction

1.1. Background

1.1.1. Definition of bioequivalence and bioequivalence studies

Health care systems use two types of drug products to maintain a sufficient supply of quality medicines to the public: innovator products, which contain new active pharmaceutical ingredients (API), and generic products, which contain the same API in a comparable dosage form. Ideally, those two types of products are interchangeable with each other. Interchangeability, also referred to as therapeutic equivalence, is established when both products are pharmaceutically equivalent and bioequivalent. Pharmaceutical equivalence of both products requires the same API manufactured in the same type of dosage form, which must be intended for the same route of administration and must meet the same quality characteristics. If those pharmaceutical equivalents show the same bioavailability, i.e. the API is available at the same rate and to the same extent at the site of drug action after administration of the same molar dose, they can be considered bioequivalent.

Bioequivalence (BE) of two products can be demonstrated by several types of studies, e.g. pharmacokinetic studies, pharmacodynamic studies, clinical trials or *in vitro* studies.[1] Pharmacokinetic studies determine and compare the concentration/time profile of an API or its active moiety in blood, plasma or serum (or other suitable fluids, e.g. urine) based on pharmacokinetic benchmarks, i.e. area under the curve (AUC) and maximum plasma concentration (c_{max}). These are considered to be the “gold standard” for BE studies.

Pharmacokinetic BE studies in humans could potentially be waived, if the bioavailability (as the basic parameter for bioequivalence) can be determined in another way. The fraction absorbed of an orally administered API, a key component of the bioavailability, can be defined as the extent and rate of absorption of that

API, without regard to metabolic effects i.e. the first-pass effect. If the absorption is mainly dependent on the dissolution of the drug product, evaluation of this dissolution behavior might serve as surrogate for the determination of bioequivalence.

1.1.2. The Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Drug Classification Scheme (BCS) was established in 1995 by Amidon et al.[2] The scheme allowed classification of APIs according to their solubility and permeability characteristics and thereby prediction of whether *in vitro* dissolution might correlate with the *in vivo* absorption behavior (*in vitro-in vivo* correlation, IVIVC) for the first time. For certain combinations, the BCS suggests that the bioequivalence of a generic product to a reference product containing the same API, could be demonstrated with an *in vitro* approach instead of pharmacokinetic *in vivo* studies.

Pharmacokinetic bioequivalence studies aim to determine the *in vivo* bioavailability, i.e. the rate and extent of absorption of an API. Solubility and permeability, two characteristics of an API itself, are considered to be the key underlying parameters of absorption.[2] Generally, an immediate release solid oral dosage form of an API disintegrates in the upper gastrointestinal (GI) tract; small particles dissolve into finer particles and finally, if it is sufficiently soluble, the API goes into solution. The dissolved API is then absorbed across the intestinal wall to an extent that depends on its permeability. Hence, information about the solubility and permeability properties of an API, in combination with details of the dissolution rate of the drug product, enable an assessment of the degree to which *in vitro* results will correlate with the *in vivo* performance of the drug product.

The classification of drugs according to their solubility and permeability is depicted in figure 1. Class I and III drugs are highly soluble, while Class II and IV drugs are not highly soluble. On the other hand, Class I and II drugs are highly permeable, while Class III and IV drugs are not highly permeable. In addition to the classification scheme, the authors provided an overview of potential *in vitro*-

in vivo correlations for the different BCS classes and provided recommendations on dissolution requirements.[2]

BCS Class I drugs are highly soluble and highly permeable; thus, they should be well absorbed. In fact, the rate-limiting step for the absorption of Class I APIs can be either the dissolution or, if the dissolution is very rapid, gastric emptying. In the former case, where the dissolution rate is the limiting factor for the absorption rate, an IVIVC can be expected. If gastric emptying is rate-determining for the absorption process, an IVIVC based on the dissolution rate cannot be established or the correlation will be limited. In this case, Amidon et al. suggest a simplified dissolution specification based on the physiological gastric emptying rate in the fasted state for a decision on bioequivalence. Since Class III APIs are highly soluble but not highly permeable, the rate of uptake into the intestinal mucosa is more likely to be rate-limiting to absorption than the dissolution rate from the drug product for these APIs. Although an IVIVC is unlikely in this case, a fast dissolution according to the simplified dissolution specification would allow a prediction of the *in vivo* performance.

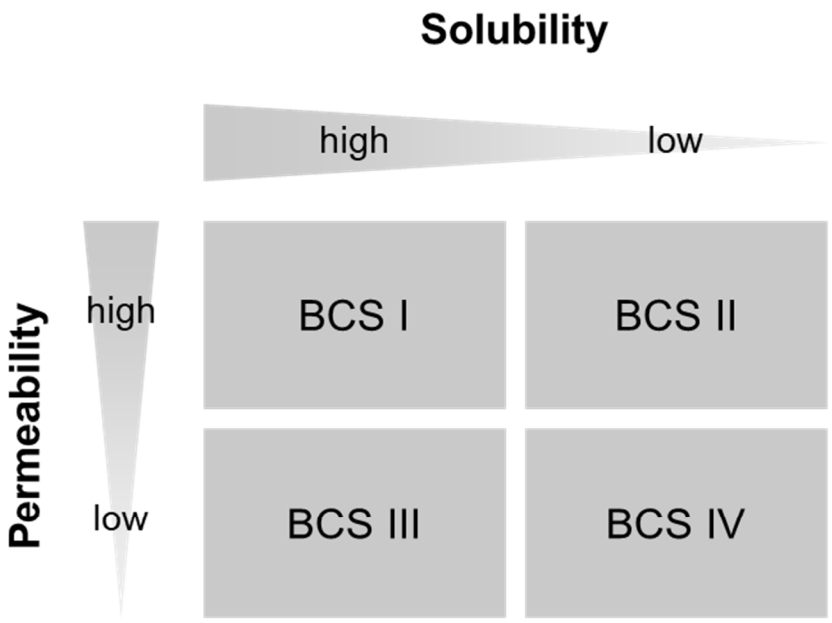

Figure 1. The Biopharmaceutics Classification System (BCS).

Definitions of a high/low solubility and a high/low permeability were not made in the original publication introducing the BCS. However, “highly soluble” and “highly permeable” were defined shortly afterwards. In the context of formulation changes with an impact on the product quality, the United States Food and Drug Administration (US-FDA or FDA) “Guidance for Industry - Scale-up and Post-Approval Changes” (SUPAC) defined “high solubility” as a dose/solubility volume of or less than 250 ml, regarding the highest approved dose strength as the “dose”.[3] An extent of absorption of more than 90% was stipulated as the criterion for “high permeability”. Later, the European Medicines Agency (EMA, formerly The European Agency for the Evaluation of Medicinal Products = EMEA), published similar definitions for “variations”, which also refers to reformulation of marketed products.[4]

With the publication of the SUPAC guidance, the FDA was the first regulatory authority to adopt the concept of the BCS. A few years later, the FDA, EMA and World Health Organization (WHO) all published (biowaiver) guidelines with detailed explanations on the cut-off criteria for solubility and permeability classification and applied the BCS concept to the approval of generic formulations using the “biowaiver” approach.[4–9]

1.1.3. BCS-based biowaiver approach

The publication of the SUPAC guidance was an initial step in the development of the “BCS-based biowaiver” approach, since changes to the manufacturing process or variations on a product formulation no longer necessarily required data from an *in vivo* bioequivalence study for a health authority approval.[3, 6, 8, 9] In parallel, the concept “biowaiver of strength”,[8] which was introduced by the EMA and WHO,[4, 7] facilitated the approval of different dosage strengths of an active pharmaceutical ingredient in immediate release oral dosage forms without pharmacokinetic bioequivalence studies for every strength. Based on the investigation of only one (reasonably chosen) strength *in vivo*, other strengths could be approved with this biowaiver procedure, provided that the drug product showed dose-proportionality.

The starting point for a BCS-based biowaiver is the classification of an API according to the BCS, since the BCS classes and their characteristics indicate the potential for oral absorption of an API from an immediate release drug product and can thus serve as a basis for the assessment of the bioavailability and bioequivalence.[2] Currently, only highly soluble drugs (BCS Class I and III drugs) are eligible for the procedure,[10–13] since a high solubility (and a rapid dissolution) minimizes absorption issues and therefore increases the reliability of the surrogate approach for bioequivalence decision-making. For a positive decision, two products containing the same API must show the same API concentration/time profile at the site of absorption. This requires the *in vivo* dissolution to be the same.[2] Provided that dissolution and absorption conditions are not affected by other factors, e.g. degradation in the digestive tract, precipitation or other sites of absorption, the *in vitro* dissolution performance of a drug product can serve as a surrogate for its *in vivo* dissolution and absorption.

Therefore, dissolution studies performed in the context of the BCS-based biowaiver should simulate the dissolution of an orally administered immediate release solid drug product i.e. a tablet or a capsule, in the human upper GI tract in the fasted state. The conditions of these studies are chosen in order to enable an *in vitro-in vivo* link and especially to distinguish the dissolution behavior of drug products with different dissolution characteristics. First of all, the volumes for the dissolution tests should maintain sink conditions, i.e. the entire dose of the API should be dissolved in one third or less of the medium volume.[2] In addition, the medium volume should be comparable to the physiological volumes together with the liquids ingested to facilitate administration of the drug product. The tests should be performed in aqueous media at pH values which reflect conditions in the different sections of the fasted gastrointestinal tract to which the drug product is exposed, e.g. pH 1 or 1.2 to simulate the gastric conditions and pH 6.8 to reflect the conditions at the main absorption sites, which are located in the upper small intestine.[10, 12, 13] Since some APIs may have a solubility minimum between pH 1.2 and 6.8 which might be a critical issue during the transit from stomach to upper small intestine, tests at pH 4.5 are additionally required. The chosen stirring speed and the temperature of the media should also reflect the *in vivo* conditions.[10, 12, 13] The dissolved amount of drug must be determined at different time

points to obtain an informative dissolution profile. For APIs whose absorption rate is determined by the gastric emptying rate or their permeability, a simplified dissolution approach showing that 85 % or more of the API is dissolved in 15 minutes ("very rapid dissolution") provides proof that dissolution is not rate-limiting to absorption and enables a decision on bioequivalence. It is required for BCS Class III APIs, but can also be applied to BCS Class I APIs. Alternatively, for Class I APIs, a dissolution of 85 % or more of the API in 30 minutes ("rapid dissolution") is sufficient. A conclusive decision on bioequivalence of two products containing a Class I API and from which 85% of the drug is dissolved within 30 minutes can be reached if the f_2-test is subsequently applied to compare the similarity of the dissolution profiles.[2, 10, 12, 13]

Hence, the solubility and permeability classifications are crucial to a decision of the suitability of an API for the BCS-based biowaiver and the dissolution data for the innovator and generic drug products are key to assessing the bioequivalence of the generic product without the need for studies in humans. However, not only the BCS class and dissolution data, but also the therapeutic range of the API must also be taken into consideration when evaluating its suitability for a BCS-based biowaiver. APIs with a narrow therapeutic index may not be considered for a biowaiver-based generic application, at least by the FDA.[10] Following this line of reasoning, the risks associated with approving a product which meets the requirements of the biowaiver procedure, but which may reach toxic concentrations *in vivo* if the biowaiver decision is incorrect, can be avoided.

In general, the potential risks associated with the approval of a drug product using the BCS-based biowaiver approach need to be evaluated in a risk-benefit analysis.[12, 13] This key element of the biowaiver procedure addresses various aspects. Risks for the public and individual patient health due to supra- or subtherapeutic levels must be evaluated and justified. Furthermore, existing reports of bioinequivalence of generic products are compelling arguments against a biowaiver-based approval. The bioinequivalence might be related to the API itself or to its formulation, in which case particular emphasis must be put on the excipients included in the manufactured product. Excipients can potentially enhance or impair

the dissolution or the absorption of the drug, therefore, they must be chosen carefully and their influence on bioequivalence must be assessed.[10, 12, 13] Only when all the above-mentioned conditions are fulfilled can dissolution data be used as a basis for the decision to allow a market authorization for a generic drug product.

1.1.4. BCS biowaiver guidelines published by health authorities

The FDA was the first health authority to publish a guidance on the BCS-based biowaiver procedure.[6] The guidance document included specifications with respect to solubility and permeability determination and classification, requirements concerning the dissolution tests to be run, mandatory considerations regarding excipients and restrictions to the BCS-based biowaiver procedure. The guidance contained some important differences to the current version.[10] Initially, the FDA allowed the biowaiver procedure only for BCS Class I APIs. The solubility data, a prerequisite for the classification, had to be determined at a pH range of 1-7.5. Additionally, the cut-off criterion for the permeability classification was different then: an extent of absorption in humans of ≥ 90 % was required.

Shortly afterwards, the EMA published a guideline covering *in vitro* bioequivalence testing with the purpose of waiving *in vivo* bioequivalence studies.[4] In comparison to the revised version that was published in 2010 and which is the currently applicable document,[12] the biowaiver procedure was not addressed as a separate subject in that guideline and it contained little detail. The requirement of the highest dose strength as basis for the solubility cut-off criterion contrasted to the current requirement to determine the dose/solubility ratio (D/S ratio) according to the highest single dose administered. Also, the pH conditions for the solubility determinations differed from the current requirements.

The WHO published a guidance document with respect to the BCS-based biowaiver approach in 2006.[9] Though the FDA guidance was used as basis for the WHO guideline, there were several essential differences between them. For example, the WHO required that the API solubility be determined over the pH range of 1.2-6.8 and that the calculation of the D/S ratio be based on the highest dose indicated in the Model List of Essential Medicines (EML). The permeability cut-off

criterion also differed from the FDA document: an API was considered to be highly permeable when ≥ 85 % is absorbed in humans. An even more important difference was the eligibility of BCS Class III and certain Class II drugs for the biowaiver procedure. APIs of Class III were required to dissolve very rapidly (≥ 85 % in 15 minutes), while Class II APIs were only eligible for the biowaiver procedure if they were weak acids with high solubility at pH 6.8. Furthermore, the WHO document contained a table that listed all APIs on the EML available in immediate release solid oral dosage forms and provided categories of their solubility, permeability, their BCS class, as well as potential recommendations for the dissolution tests to be applied.

In 2017, the recommendations for *in vitro* equivalence testing were incorporated in the guideline regarding bioequivalence evaluation for generic products.[13] In addition, the WHO published a draft protocol for conducting equilibrium solubility studies for BCS-based biowaivers in 2018.[14] Some of the key aspects of the original WHO biowaiver approach were changed with the revision of the document, such that only BCS Class I and III APIs are now eligible for the biowaiver procedure. Further, the assignment of APIs to a solubility class is based on calculations with “the highest single therapeutic dose as determined by the relevant regulatory authority”, which is comparable to the approach of the EMA.

The WHO guideline is intended to serve as a basis for decisions on biowaiver applications of national regulatory authorities worldwide.[9] It could be used as guidance document or could be implemented in national law, which is why the biowaiver procedure has attained broad international recognition. The same applies for the EMA guideline, since it is applicable in all states of the European Union. Indeed, many countries adopted the approach and have implemented one of the three guidelines (FDA, EMA, WHO) or published their own criteria for BCS-based biowaivers. For example, Argentina,[15] the ASEAN states,[16] Australia,[17] Brazil,[18] Canada,[19] Malaysia,[20] New Zealand,[21] Singapore,[22] South Africa,[23] Switzerland,[24] and Thailand[25] all allow biowaiver applications under certain conditions. Since the BCS-based biowaiver is now of global interest and because there is still a lack of harmonization between the guidelines of the three health author-

ities FDA, EMA and WHO, e.g. regarding the dose which should be the calculation basis for the solubility classification, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) provided a draft guideline for public consultation (a multipurpose guideline, M9) in order to harmonize the details of the procedure and enable the implementation in all ICH countries.[26] Since this guideline was not finalized until late 2019, it will not be discussed further in this dissertation.

1.1.5. Biowaiver monograph series

One of the focus groups of the International Pharmaceutical Federation (FIP) special interest group “Regulatory Sciences and Quality” is the group “Bioclassification/Biowaiver”.[27] With the publication of a commentary on the applicability of the biowaiver on verapamil hydrochloride, propranolol hydrochloride, and atenolol in 2004, the focus group started a publication project which became known as the biowaiver monograph series.[28, 29] The main goal of the project was to provide all relevant information for individual APIs that are either listed on the EML or are in widespread use and to scientifically review these data in the light of a potential recommendation for the BCS-based biowaiver procedure. The summary of all reviewed information – the monograph – could be used as a starting point to apply for a generic drug approval by the health authorities.[29] The need for such a case by case project resulted from the regulatory situation of the BCS biowaiver procedure. The procedure had already been implemented through the publication of FDA and EMA guidelines which established the BCS-based biowaiver and presented the requirements for an application.[4–6] However, the guidelines were not harmonized, a situation which was exacerbated by the publication of the WHO biowaiver guideline in 2006.[9] Furthermore, the regulatory documents described general prerequisites and *in vitro* methods which should be applied to obtain the required data, but no concrete information about the eligibility of individual APIs and the suitability of their drug products for a BCS-based biowaiver was communicated.[29] The publication of monographs for individual APIs including relevant data and their assessment leading to a recommendation in terms of a BCS biowaiver, was a logical consequence of this gap.

A biowaiver monograph should provide all data which are available from the open pharmaceutical literature to characterize the drug in general (e.g. molecular structure, indication, therapeutic index, toxicity) and with respect to its physicochemical and pharmacokinetic properties (physicochemical properties could include solid and especially polymorphic forms, stereochemistry, acidic/basic properties, partition/distribution coefficient, solubility and stability, while pharmacokinetic properties comprise absorption, bioavailability, permeability, distribution, metabolism, and elimination).[30, 31] Additionally, information on existing dosage forms and their performance (bioequivalence/bioinequivalence reports, dissolution results, and excipients) should be included.[30, 31] Whereas at the beginning of the biowaiver project, data for solubility were extracted solely from the literature and discussed in terms of their physiological relevance,[28] a lack of solubility data at pH values of interest is now addressed by additional solubility studies.[29] Dissolution data of the API and - if possible - reference and generic products can also be generated and reported in the monograph. An assessment of the collected data is of central importance, not only with regard to the BCS Class and the therapeutic index, which are the basis for API eligibility, but also for a complete risk assessment.[29] The risk considerations should contain a careful evaluation of the risk for products not to be bioequivalent due to excipients and/or the manufacturing process, as well as an assessment of the risks of a false-positive biowaiver approval for patients and for public health.[29–31] The final assessment of the monograph should also identify gaps in the existing data. If a positive decision is reached about suitability of the biowaiver procedure, the monograph recommends testing conditions and requirements for a biowaiver application (e.g. stability-indicating dissolution tests).[32]

The evaluation itself and any recommendations for a specific API should follow best scientific practices, rather than merely checking compatibility with the existing regulatory documents.[29] This idea of a scientific debate for each API opened up the possibility for the biowaiver monographs to be a platform of scientific discussion, which in turn became a leading motivation for harmonization of the health authority guidelines.

Another objective of the project was to provide a scientifically based source of information for regulatory authorities and applicants.

Up to the present day, monographs for 52 APIs have been published under the auspices of the FIP focus group “Bioclassification/Biowaiver”.[33] They are published in the Journal of Pharmaceutical Sciences and are also available at the FIP website free of charge (https://www.fip.org/bcs-monographs).[33] The primary intention of the biowaiver monograph project was and still is to prepare and publish monographs of APIs which are published on the WHO Model List of Essential Medicines as immediate release solid oral dosage forms.[29, 34]

1.1.6. The Model List of Essential Medicines

The term “essential medicines“ is defined by the World Health Organization. According to the definition, essential medicines meet the major health care needs of the population and are selected the on basis of their relevance to public health and evidence of their efficacy, safety, and cost effectiveness.[35] All essential medicines are summarized and published on the Model List of Essential Medicines, which was published for the first time in 1977.[34] The list with the selected drugs and their recommended dose in usual dosage forms has been updated approximately every two years since the publication of the initial volume. The list is currently available in its 21st version and can be accessed online (https://apps.who.int/iris/rest/bitstreams/1237479/retrieve).[36]

Though the EML is not binding, it serves to provide recommendations for every health care system to ensure the availability of essential medicines in an appropriate quality and quantity and at affordable costs. Further, the continuous revision of the list plays an important role with respect to the prevention of (global) drug resistance.

The publication consists of a core list containing the minimum standard medicines and a complementary list, containing medicines for which special conditions, e.g. those regarding diagnosis or monitoring, are required. Additionally, an independent list of essential medicines for children was published by the WHO in 2007[37]

and is now available in its 7th edition[38]. The children's list comprises medicines for the basic medical treatment of pediatric patients i.e. children below the age of twelve.

1.1.7. Definitions of solubility

Specific definitions of solubility vary. In general, solubility is a chemical property of a substance and describes the extent of homogenous distribution of this substance in another substance. The International Union of Pure and Applied Chemistry (IUPAC) defines solubility as the composition of a saturated solution which is expressed as the proportion of solute to solvent.[39] It can be expressed as a (mass) concentration, but according to IUPAC, also as a molality, mole fraction or mole ratio.[39] Other sources define additionally the temperature at which the saturated concentration is determined.[40] The following section addresses solubility definitions of solid substances in liquids, especially aqueous solutions, though in general, a solute can be gaseous, liquid or solid and the solvent can be liquid or solid.

The thermodynamic solubility is also known as the equilibrium solubility. If a solid is added to a solvent, for example water, it dissolves with a certain rate. This dissolution rate can be determined according to the equation of Noyes and Whitney[41] or Nernst and Brunner,[42, 43] who modified the Noyes-Whitney equation (equation 1). Given that enough solid material is available, the dissolution process continues until the solution is saturated with the solute. At this saturated state, an equilibrium is reached; further solid substance will dissolve with the same rate and to the same extent as the dissolved substance will precipitate. Most definitions of solubility refer to this chemical state.

Eq. 1

$$\frac{dC}{dt} = \frac{D \times S}{V \times h} (C_S - C)$$

d*C*/d*t*	Amount of dissolving substance per unit of time
D	Diffusion coefficient
S	Surface area of solid particle
Cs	Saturation solubility

C Current concentration of dissolved material in solvent
V Volume of the dissolution medium
h thickness of diffusion layer

If and to what extent the dissolution reaction will run, is determined by the reaction´s free enthalpy or Gibbs energy, which must be negative (equation 2). Enthalpy (H) is a function of state and describes changes of energy due to volume work of a system. The change in enthalpy (ΔH) is equal to the amount of energy which is emitted or absorbed in the form of thermal energy and can be quantified. Negative values for ΔH describe exothermic reactions, while positive values describe endothermic reactions.

Eq. 2
$$\Delta G_{mix} = \Delta H_{mix} - T \times \Delta S$$

ΔG_{mix} Gibbs free energy of mixing
ΔH_{mix} Enthalpy of mixing
T Temperature
ΔS Entropy of mixing

The enthalpy of mixing refers in this case to the opening and forming of chemical bonds during the dissolution process which is accompanied by a change of energy e.g. opening of the bonds in the solid material (lattice energy), establishing hydrogen bonds with water (solvation energy), and reestablishing water clusters. According to the Gibbs equation, the free energy change is also determined by the entropy, which is positive for mixing processes. Entropy (S) describes the degree of disorder of a system; it increases with the degree of disorder in every isolated system. Like enthalpy, the entropy itself is not directly quantifiable, only the change in entropy (ΔS). Regarding entropy changes for the mixing or dissolution process, the most relevant entropy is the mixing of the solute and solvent molecules which increases the degree of disorder.

The thermodynamic solubility of a substance in water depends not only on the temperature, but also on the pressure and can be influenced by the pH (of an aqueous solvent) and other physicochemical effects e.g. modification of crystallinity, solubilization via a third party substance, and the use of cosolvents.

In contrast to the thermodynamic solubility, the “kinetic” solubility describes a metastable state. An example is when the solute dissolves, but then precipitates in a less stable polymorph or amorphous form. Although solubility studies for BCS purposes generally aim to measure the thermodynamic solubility, in certain circumstances the kinetic solubility may be measured instead.

1.1.8. Solubility determination methods

Several methods to determine the solubility of APIs are available for measuring the thermodynamic solubility. They can be divided in analytic, synthetic and indirect methods.[44] Analytic methods determine the concentration of a saturated solution at a certain temperature and are among the earliest methods described in the literature.[41, 45] Indirect methods use physical parameters like electric conductivity, electric potential, density, or refraction index for the determination of the solubility.[44] Changes in the physical parameter indicate a change in solubility. The following section provides examples for the most common analytic and indirect methods and also presents a method which is suitable to determine the kinetic solubility.

Pharmacopoeial methods to define the solubility are analytic methods which are based on visible results e.g. the solubility determination according to the European Pharmacopoeia.[40] A defined amount of API (initially 100 mg) is mixed with a defined volume of water (initially 0.1 ml) at a certain temperature (25 ± 0.5 °C). To classify the substance in terms of solubility, the mixture is visually examined. Depending on how many times another aliquot of water must be added to completely dissolve the powder, it is assigned to an appropriate solubility category, e.g. if 100 mg of the API are dissolved in 0.1 ml of water, the API is classified as “very soluble” whereas a dissolution of those 100 mg in 10 ml of water classifies the API as “sparingly soluble”.[40] Repetitions with a smaller amount of API (e.g. 10 mg) and a higher volume of water (e.g. 10 ml) are necessary for poorly soluble APIs.

Another analytic method is the shake-flask method, which is the classic method to determine the equilibrium solubility. For a determination with this method, the

API powder is transferred into a flask and the test medium (e.g. water, buffer solution, etc.) is added (e.g. 250 ml). Ideally, the amount of powder constitutes an excess of API with respect to the volume of medium and thus results in a saturated solution. The amount required to create an excess can be determined in pilot experiments. The flask is then shaken, which helps to distribute the powder, to avoid wettability problems and bring the API into solution. The shaking time can be defined in advance or determined during the experiment but should be long enough to establish equilibrium. During the experiment, the temperature should be held constant. If a certain pH value is required, the pH can be adjusted during the shaking time if the buffer capacity of the medium proves insufficient i.e. the pH changes during the experiment. After shaking, the solution is filtered to separate undissolved particles from the solution and the API concentration in the filtrate is quantified. With this method, the thermodynamic solubility of the most stable form can be determined at a defined pH and a defined temperature.

A commonly used indirect solubility determination method is the potentiometric titration. This method is based on the pH-profile of an API obtained from an acid-base titration and the characteristic shift of the titration curve due to precipitation beginning at a certain pH value.[46] It is applicable to ionizable APIs with known pK_a values and ideally a known log P value.[47] The titration can be performed using an automated instrument, which calculates the estimated intrinsic solubility and simulates the titration curve based on those data in advance to the experiment. In addition, a blank titration is required prior to the experiment. The API is accurately weighed and dissolved in water; a cosolvent might be added if necessary.[48] The accuracy of the titration can be improved by the addition of potassium chloride and the use of inert gas.[46] Starting the formal titration, strong acid or base is added to the API solution in predefined volume steps during which the pH of the solution is constantly monitored with an electrode that detects the potential difference. Thereby, the pH profile is recorded throughout the experiment. Usually, acidimetric titrations are used for acidic APIs and alkalimetric titrations for basic APIs. Approaching the pK_a of the API, its ionization is reduced, resulting in precipitation. To avoid supersaturation of the uncharged API, the titration might start from precipitation in the direction of dissolution, i.e. the API might first be dissolved in strong acid or strong base and then precipitated again (in advance

of the formal titration).[47] Figure 2 depicts a fictional titration curve of a weak base showing precipitation during the titration process (negative equivalents refer to an acid titrant, whereas positive equivalents refer to a basic titrant).

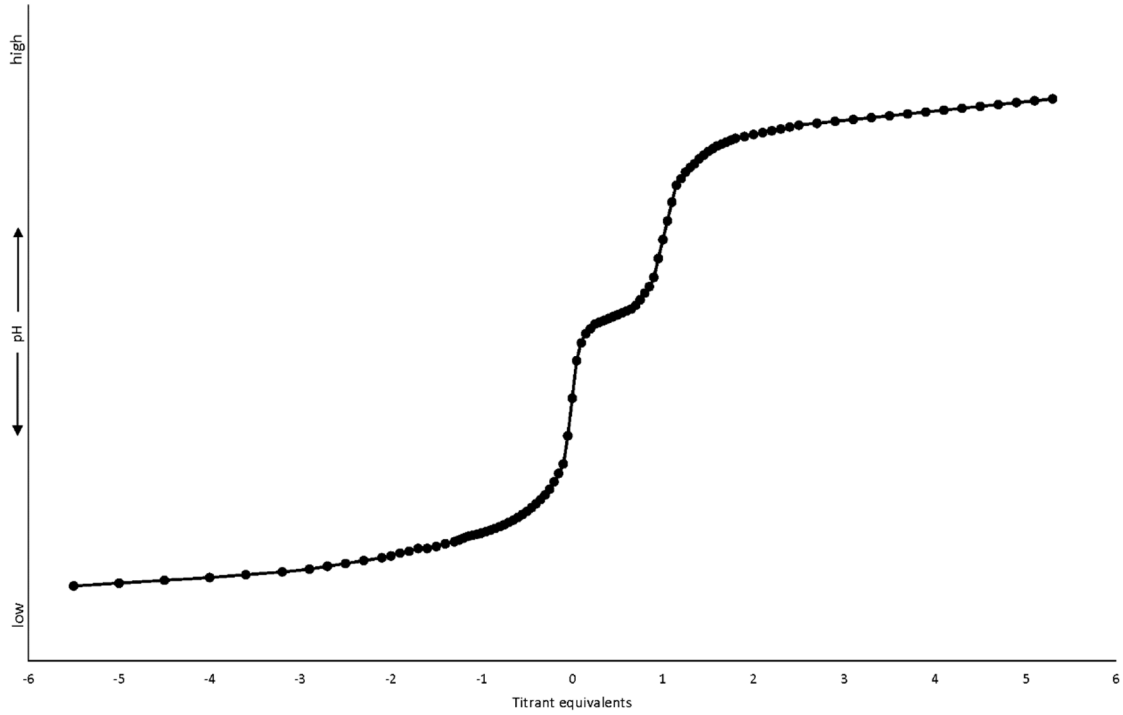

Figure 2. Fictional titration curve of a weak base showing precipitation during alkalimetric titration.

Normally, the pK_a of a weak acid or base is shown at the half equivalence point, which is the inflexion point of the titration curve with the lowest slope. Due to the precipitation, the titration curve for the solubility determination shows an additional inflection point (indicating the starting point of precipitation) and the pK_a derived from such a curve differs from the real value.[48] A large difference between the real pK_a value and this apparent pK_a value indicates that the solubility of the API is low.[46] To determine this difference and evaluate the solubility exactly, the Bjerrum difference plot, which plots the average number of bound protons ($\bar{n}_H$) against the pH, is used.[48] It can either be obtained by mathematical calculation or by the difference of the sample titration and the blank titration. If the difference between the real pK_a and the observed, apparent pK_a is calculated with the help of a Bjerrum plot, the solubility can be calculated with the following equation:[46, 48]

Eq. 3

$$\log S_0 = \log(\frac{C}{2}) - |pK_a^{app} - pK_a|$$

C	Concentration of the substance solution
S_0	Intrinsic solubility of the unchanged substance
pK_a^{app}	Measured pK_a value of the substance with precipitation
pK_a	Measured pK_a value of the substance without precipitation

Further calculations allow the construction of a pH-solubility profile.[48] Although several titrations might be necessary to obtain a solubility value, the amount of API required is comparatively low.[46] However, the solubility of APIs without acidic or basic molecule groups cannot be determined.

Turbidimetry is another solubility determination method based on a titration with precipitation occurring during the titration process, but in contrast to the potentiometric titration, it can result in a kinetic solubility determination.[46] The method is based on precipitation of the API during titration, which results from an exceeded equilibrium of dissolved and undissolved API. The progress of precipitation is detected by measuring the increasing turbidity of the solution. In preparation for a turbidimetric solubility determination, the API is dissolved in an organic solvent, dimethyl sulfoxide (DMSO).[49] This solution is gradually added to a buffer solution with a given pH. Between the addition intervals, the turbidity of the mixture is measured by light scattering.[46] The measured values are plotted against the volume of the organic solution to determine the starting point of precipitation by extrapolating to zero turbidity and therefore the solubility of the API.[49] DMSO, however, may facilitate supersaturation, such that the solubility measured is higher than the real value,[46] since the precipitate is not necessarily the thermodynamically most stable form. Under these circumstances a kinetic solubility will be measured. Turbidimetric determinations are thus typically used to screen many substances for an estimate of solubility in a medium to high throughput setting, rather than to provide exact measurements of the thermodynamic solubility.

1.1.9. Potential issues with solubility in the context of BCS biowaivers

The solubility determination of the API is a fundamental pillar of the BCS-based biowaiver procedure. Several factors with respect to the setup of solubility studies to obtain these data should be taken into consideration in the context of BCS-based biowaivers and also biowaiver monographs. These are described in the following sections.

1.1.9.1. Physiological relevance of solubility data

The solubility of an API, along with other physicochemical properties, is usually evaluated during pharmaceutical development using conventional methods described by the United States Pharmacopeia (USP) or the European Pharmacopoeia (Ph. Eur.). Solubility measurements are usually performed in water (among other solvents).[40, 50] In this way, the solubility characteristics of different APIs are comparable. Many equilibrium solubility values reported in the open literature have been obtained from experiments in water at room temperature. However, the solubility data for a BCS classification must be obtained under physiologically more relevant conditions. The test media should reflect the pH conditions to which an API is exposed after oral administration in the GI tract.[10, 12, 13] In addition, solubility determinations for BCS purposes must be conducted at 37 °C, the human core body temperature. The duration of the solubility experiments is not defined by the guidances, but a period corresponding to at least the passage time of an orally administered API though the upper gastrointestinal tract is a reasonable lower limit.

Nevertheless, solubility values from experiments in water at room temperature provide a first estimate of the solubility of an API in the fluids of the human GI tract. In general, regarding the correlation of the *in vitro* solubility results of a API obtained in the context of BCS-based biowaivers with the *in vivo* solubility of the same API, it should be kept in mind that APIs can build a supersaturated solution during passage through the gastrointestinal tract (either due to formulation effects

or if the drug is a weak base and thus far more soluble under gastric than intestinal conditions), but then precipitate later in another, less stable solid form which may have a higher solubility.[51]

1.1.9.2. Analytical aspects

Since most of the BCS biowaiver guidelines require the determination of the equilibrium solubility, turbidimetry or other methods that determine the kinetic solubility are not deemed suitable for the setup of a solubility study.[10, 12–14] Traditionally, the shake-flask method is recommended for solubility determinations, but with appropriate justification, other methods such as acid-base titrations can also be used.[10, 12-14] Although potentiometric acid-base titrations only require small amounts of API, they can only be applied to substances which have acidic/basic functions with known pK_a values. Therefore, the shake-flask method has a broader scope of application and is considered to be the more suitable method. The use of a scaled-down approach is possible to minimize the necessary amounts of API. In 2005, Glomme et al. compared the results of a miniaturized i.e. scaled-down shake-flask method with those of the conventional shake-flask method and demonstrated that reliable results can be obtained using the scaled-down version.[52]

While requirements or recommendations for the solubility determination methods have been published in the biowaiver guidelines, limited information about suitable quantification methods is available. Ideally, a quantification method is simple, cost-effective and easily validated. A popular method that fulfills the criteria of efficiency and economic considerations is ultraviolet (UV) spectroscopy. A disadvantage of this method is the lack of specificity for the API. Degradation products of the API, formulation excipients and components of the medium in which the solubility is being tested are possible sources of interference with the UV absorbance of the API at the chosen wavelength of measurement.

The use of a high-performance liquid chromatography (HPLC) method is generally more time-consuming and cost-intensive than UV analysis. However, HPLC

analysis has the advantages of separating degradation products and other substances which may interfere with the detection of the API and thus enabling unequivocal quantification of the API concentration. The WHO guideline, for example, recommends the use of a pharmacopoeial HPLC method for analysis whenever this is available.

1.1.9.3. Stability problems of the test substance

Some APIs are unstable under the pH conditions or at the temperature required for solubility studies for BCS-based biowaivers. Stability issues can also arise due to other aspects of ambient conditions e.g. light, oxygen, or humidity, during preparation for a stability study or during sample handling. As mentioned, it should be possible to detect degradation products with the analytical method so that their impact on the solubility and permeability results and therefore the BCS classification can be evaluated and discussed. Thus, the biowaiver guidance documents published by the FDA and the WHO require that the analytical method for the determination of solubility results is "stability-indicating".[10, 13] Ideally, the quality "stability-indicating" should not only describe a method with which stability and degradation issues are detected, but which also avoids instability during the analysis. Preferably, a solubility study should be designed to ensure the stability of the individual API to be tested, under the solubility test conditions and in the samples to be quantified during the analysis phase of the study. Therefore, a literature search that comprises stability reports and analytical methods with reproducible results (e.g. analysis methods published in the different pharmacopoeias) should be performed in advance of the solubility study. The information retrieved is then used to design the solubility studies so that the exposition of the API to stress factors is minimized, e.g. the use of amber-glass vials for photosensitive APIs or short sample handling times.

1.1.9.4. Definitions of dose according to different guidelines

The FDA, EMA and WHO biowaiver guidelines classify drugs as "highly soluble" or "not highly soluble" according to the dose/solubility (D/S) ratio.[10, 12, 13] However,

the definition of dose differs among the biowaiver documents of the various health authorities as discussed in previous paragraphs. Several definitions, including “highest (dose) strength”,[10] “highest single dose administered”,[12] and “highest single therapeutic dose as determined by the relevant regulatory authority”[13] can be found in the guidance literature, highlighting the lack of harmonization. For guideline-conform solubility studies, all definitions must be considered, but at least the highest dose resulting from those definitions should be used for a worst-case calculation. The solubility must be obtained at equilibrium,[10, 13] i.e. when the medium is saturated with the API and solid API continues to be present after an adequately long time frame.

In some cases, the entire dose of a poorly soluble API in a volume of 250 ml of medium will create a saturated solution, allowing the solubility to be determined at equilibrium. Alternatively, if the entire dose of an API dissolves in 250 ml of media over the required pH range, this could be considered as sufficient evidence for a classification as “highly soluble”. However, since the guidances stipulate that the equilibrium solubility nevertheless must be determined, large amounts of API may be required to determine the solubility of very soluble drugs. In turn, this can potentially induce problems such as being unable to maintain a constant pH in the solution, as well as high material costs.

1.1.9.5. Economic aspects

Among the various APIs listed on the WHO EML, are several that are inexpensive (e.g. nitrofurantoin) and others that are comparatively expensive (for example ritonavir). APIs that are still under patent protection and/or whose drug design is complex are especially likely to be expensive. If the solubility and dissolution data required for a BCS biowaiver submission are not available in the open pharmaceutical literature or if the data are incomplete, studies to obtain these data must be run. For the determination of the solubility in the context of the BCS-based biowaiver, the amount of API powder is determined, *inter alia*, by the dose of the API. The biowaiver guidelines require the evaluation of either the highest dosage strength of a drug product, in which the API is already available on the market, or

the highest single therapeutic dose, which can be an even higher amount. Nitrofurantoin and ritonavir, for example, are both available in 100 mg solid oral dosage forms, but ritonavir can be given in singles doses of up to 600 mg. For the determination of the equilibrium solubility, an excess of substance must be used in order to provide a saturated solution of the particular API. If the traditional shake-flask method is used, large amounts of API may be needed, especially if the drug is very soluble. If the solubility is not known and literature data do not allow a rough estimation, pilot studies should be run to determine approximately how much API will be required to saturate the media. Several pH conditions must be evaluated in the formal solubility study and multiple samples for each condition need to be prepared and tested. An additional amount of API will be required if a HPLC method for the quantitative analysis needs to be developed and validated.

Hence, the amount of powder for pilot and formal solubility studies to determine the equilibrium solubility for a BCS-based biowaiver can be quite high, leading to high costs as well as potentially to environmental contamination, if not properly disposed. However, the amount of API can be reduced by using the scaled-down shake-flask approach published by Glomme et al.[52]

1.2. Aim of the thesis

The above-mentioned biowaiver monograph series aims at providing a scientific evaluation with respect to eligibility for approval via the BCS biowaiver procedure for APIs listed on the WHO EML. In collaboration with the FIP Focus Group Bioclassification/Biowaiver, the Institute of Pharmaceutical Technology at the Goethe University, Frankfurt, under the direction of Prof. Dressman prepares biowaiver monographs for antimalarials, antiretrovirals, anti-infectives and other APIs which are listed as solid oral dosage forms on the EML.

The first objective of the present work was the continuation of this project. Two APIs were selected as candidates for a biowaiver monograph, proguanil hydrochloride, an antimalarial which is primarily used for prophylaxis in combination products, and cefalexin monohydrate, an anti-infective agent of the cephalosporin antibiotic class, both of which required special considerations regarding the design of solubility studies due to their physicochemical characteristics.

A second objective of this work resulted from a project that was initiated by the WHO to determine the solubility of newly added APIs of the 16th and 17th version of the EML at pH values of 1.2, 4.5 and 6.8. The high number and in some cases the high costs of the test APIs required a cost-effective and simple study design that could also be used for the solubility characterization of future new compounds on the biennially updated EML.

Both projects illustrated the necessity of an optimized design for solubility studies which is effective and affordable, provides scientifically reliable solubility data and enables the evaluation of substances with stability issues. Therefore, the overall objective of this thesis was to establish an appropriate solubility determination approach, including a study protocol, for solubility studies performed in the context of biowaiver monographs.

2. Results and Discussion

2.1. Requirements for solubility determinations for a BCS biowaiver monograph

Solubility studies performed in 392the context of BCS biowaiver monographs must distinguish whether an API fulfills the BCS definition of “highly soluble” or not. Since the biowaiver approval procedure can currently only be applied to highly soluble, i.e. BCS class I and III, APIs,[10, 12, 13] a classification of “not highly soluble” will exclude a drug candidate from the procedure.

The cut-off criterion for a solubility classification cannot be found directly in the original publication of the Biopharmaceutics Classification System but can be obtained from the guidelines of the health authorities FDA, WHO, and EMA. The US FDA SUPAC guidance, which was the first document that adopted the BCS as scientific basis for an abbreviated approval procedure, defined a D/S ratio of ≤ 250 ml in the physiological pH range for highly soluble drugs.[3] The dose for the calculation of this ratio was defined as the highest dosage strength of manufactured products of an API. With the publication of the first biowaiver guidance in the year 2000, the FDA required a D/S calculation based on “the highest strength of an IR product that is the subject of a biowaiver request”.[6] This specification is also required in the draft guidance for revision in 2015 and in the published guidance from 2017.[6, 10, 53] Additionally, the 2017 guidance requests further information if the highest single dose administered exceeds the highest strength and this leads to a change in the solubility classification.[10] The EMA guideline, which addresses bioequivalence issues including considerations for products with manufacturing variations compared to the original approved formulation and which was released shortly after the first FDA biowaiver guidance, initially required “the highest dose strength” and therefore the same basis for the cut-off criterion.[4, 5] However, that definition was later changed to “the highest single dose administered as immediate release formulation(s)” with the revision of the guideline, which was published in 2010.[8, 12] The first guideline of the WHO regarding the waiving of *in vivo* bioequivalence studies allowed the procedure to be considered

for immediate release solid oral dosage forms listed on the EML. In terms of the dose, the guideline referred to the central statement of the FDA guidance ("highest orally administered dose") but revised the criterion by relating it to the EML ("highest dose indicated in the Model List of Essential Medicines (EML)").[9] During the revision process that started in 2014, the positions of both the FDA and the EMA found their way into the guidance text.[54] However, the final published document defined the dose for the D/S ratio as the "highest single therapeutic dose as determined by the relevant regulatory authority, typically defined by the labelling for the innovator product" which is in agreement with the text of the EMA guideline.[11, 13] The development of the guideline specifications regarding the dose over the past two decades illustrates the perceived importance of this parameter for the design of solubility studies for biowaiver monographs. Both approaches, the calculation with the highest dose strength and the highest single therapeutic dose, have advantages and disadvantages as discussed by Barends et al.[29] To cover all jurisdictions, dose/solubility values currently need to be calculated with the highest dose strength in which an IR product is available and with the highest single therapeutic dose which is administered of a drug product. Additionally, a calculation using the highest dose strength listed on the EML is needed to complete the solubility assessment of a biowaiver monograph for drugs listed on the EML. Often subject of a biowaiver monograph, the APIs that are listed on the EML are of special interest in this regard, since the doses recommended on the EML are often lower than those approved by the individual regulatory agencies like FDA.

A drug can only be classified as "highly soluble", if the D/S ratio is equal or below a volume of 250 ml of aqueous media (EMA: buffer) over the physiological pH range. Currently, this range is generally defined by the health authorities to be 1-6.8 (WHO: 1.2-6.8). However, the concrete values at which the solubility should be determined differ: the FDA currently requires determinations at pH 1 and 6.8, at the pH of the pK_a, and at one unit below and above the pK_a, preferably in USP standard buffer solutions. The EMA document prefers determinations at a minimum of three values, namely 1.2, 4.5, and 6.8 and in addition at the pK_a value if it is in the above-mentioned range. The WHO has a scientifically more reasonable approach requiring determination "at the pH of any known solubility minima" in

the range of 1.2-6.8 in addition to the determinations at the single values 1.2, 4.5 and 6.8. Solubility studies in the context of biowaiver monographs should therefore include tests at all mentioned pH values. For an easier but less conclusive estimation of the eligibility of a drug candidate for a biowaiver, experiments at only the key pH values (1.2, 4.5, and 6.8) can be determined.[55] These pH values are the essential pH conditions which are encountered by a solid oral dosage form passing through the upper gastrointestinal tract from the stomach (pH ~1.2) to the mid-jejunum (pH ~6.8). pH 4.5 represents a pH between these two extremes that may be useful in identifying the minimum solubility over the range pH 1-6.8. A full pH-solubility profile over the physiological pH range, however, helps to detect all possible solubility minima, minimizing the risk of a false solubility classification. Experiments at each given pH should be performed at least in triplicate. The FDA and the WHO recommend the use of pharmacopoeial buffers (USP standard buffer solutions and buffers from the International Pharmacopoeia, respectively) as aqueous media for the solubility studies. However, it must be noted that some pH values are not covered by the registers in either pharmacopoeia (see Table 2). In this case, the buffer composition can be chosen from the list of buffers in the European Pharmacopoeia. The temperature at which the solubility is determined should be 37 ± 1°C. Ideally, the pH of the buffer would be adjusted at the study temperature.[14] Since the pH value and the volume of the buffer solution at 37°C are different from those values at room temperature, an adjustment at the study temperature should be avoided if it cannot be ensured that all pH measurements and sampling and dilution of the study samples are performed at this temperature. Otherwise, pH values before and after the study are not comparable and concentrations cannot be calculated accurately.

All health authorities suggest the shake-flask method as method of choice for the determination of an (equilibrium) solubility profile. This method is associated with high material costs, especially when a high number of pH values should be tested. The shake-flask method can also be performed in a scaled-down i.e. miniaturized approach to obtain reliable solubility data.[52] For the solubility studies for proguanil and cephalexin as well as for the solubility classification of newly added compounds of the EML, the miniaturized approach was implemented by use of a

Uniprep™ filter system (depicted in figure 3),[30, 31, 55] following the Glomme approach.[52] The experiments were performed over a period of 24 hours. Though the guidance documents of FDA and WHO require the determination of the equilibrium solubility without mentioning the time frame, a duration of 24 hours is a reasonable starting point, since GI transit times including the transit through absorption compartments rarely exceed 24 hours while at the same time equilibrium can be achieved by most substances within this period.[55]

The pH of the medium might change during the study period. According to the biowaiver guidelines of the FDA and WHO, the pH of the medium should be verified after the addition of the API i.e. measured and adjusted to the target pH.[10, 13] However, an adjustment of the pH after addition of the API and during the study time is not recommended for solubility studies when using the miniaturized approach, since both the measurement itself and the adjustment would change the total volume and (therefore) the composition of the solution (e.g. water from rinsing the electrode might be brought to the solution of the sample and/or strong bases or acids could be added).[55] Both would increase the risk of variations in the sample constitution. Instead, the pH should be checked at the end of the experiments when implementing this method.

According to the current versions of the FDA and the WHO guidance regarding the biowaiver procedure, validated stability-indicating analytical methods should be used to determine the concentration of dissolved drug after the experiment. The WHO suggests a high-performance liquid chromatography analysis. The advantage of this analysis method is the capability to detect products resulting from physicochemical instabilities such as degradation, occurring from the study conditions as well as the analysis conditions. If a HPLC quantification method is not available in pharmacopoeial monographs or the open pharmaceutical literature, a new stability-indicating method must be developed and validated. The validation should be in accordance with the standards of the International Conference on Harmonisation guideline Q2(R1)[56] with a focus on linearity, limit of detection and limit of quantitation, precision, and accuracy. Since solubility studies should be carried out with the pure API, the proof of specificity plays only a minor role. According to the pharmacopoeias, system suitability testing is recommended if

possible.[57, 58] To avoid stability issues during the phase of analysis, the stability of the API under the conditions of the stability-indicating method must be examined for the duration of analysis. In general, issues such as degradation or precipitation should be identified in advance and the experimental conditions (for both the study and the quantification method) should be adjusted to minimize them. If degradation occurs, the degradation products and their resolution should be identified, so they can be reported with the solubility results, especially if it leads to a changed solubility classification.[55]

The concept, which was established on the basis of all requirements by the health authorities FDA, WHO, and EMA, was applied to the solubility studies for the biowaiver monographs of proguanil hydrochloride and cephalexin monohydrate with the aim of determining whether the D/S ratio is equal or less than a volume of 250 ml over the entire pH range.[30, 31] The solubility classification of newly added compounds of the EML was also based on those requirements, but was reduced to determinations at only the key pH values to provide information about the eligibility for the biowaiver procedure for this larger number of APIs.[55]

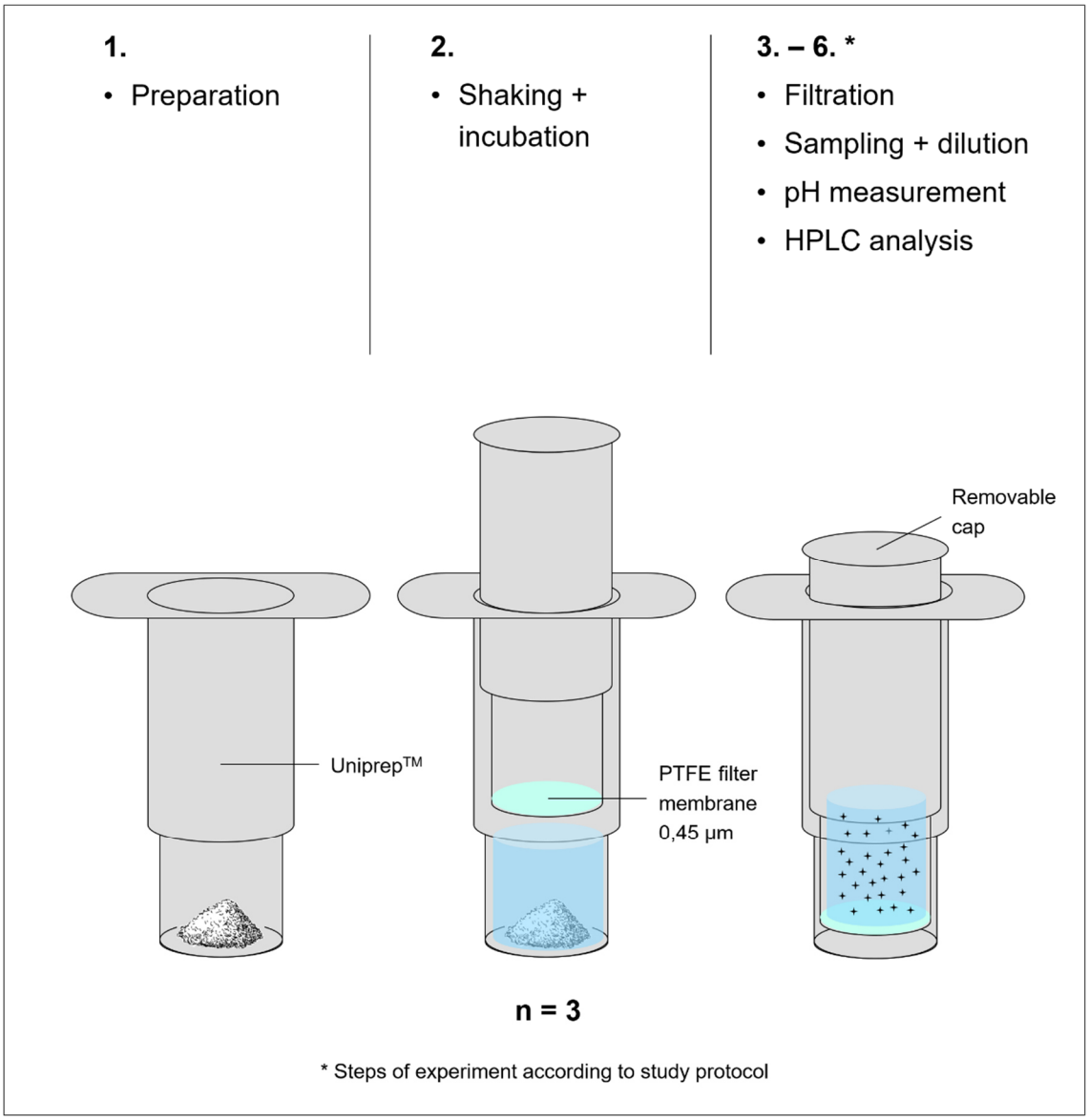

Figure 3. Solubility determination approach based on the miniaturized shake-flask method.

2.2. Study protocol for solubility studies

To standardize solubility experiments for different APIs in the context of BCS biowaiver monographs, the requirements and recommendations for solubility determinations that were discussed in the previous section are summarized into a study protocol (see Table 1). The study protocol is comparable to the published protocol in the solubility classification study regarding newly added compounds of the EML,[55] but gives more advice about how to design the experiments for biowaiver purposes. The solubility classification of proguanil hydrochloride and cephalexin monohydrate followed this protocol.[30, 31] The experimental setup is shown in figure 3.

Table 1. Study protocol for the solubility determination of APIs in the context of biowaiver monographs

Conditions	**Comments**
1. Preparation of solubility samples in Uniprep™ syringe-less filters	Prepare compendial buffers with a pH of 1, 1.2, 4.5, 6.8 and, if applicable, at the pKa, the pK_a ± 1 and at the pH of any known solubility minimum (buffer check: pH ± 0,05).
	Weigh an excess of the API into Uniprep™ vials (n ≥ 3 for each pH/buffer).
	Add three mL of the buffer solution to each Uniprep™ vial.
	Seal vials with the Uniprep™ plungers.
2. Shaking + incubation	Samples are incubated in an oven maintained at 37 ± 1 °C and shaken for 24 hours, e.g. on an orbital shaker at 45 rpm.
	Regular visual inspection is recommended.
3. Filtration	Before filtration, check the status of dissolution, i.e. whether any solid API could be visually detected.
	Push the Uniprep™ plunger into the vial to effect filtration.

Conditions	Comments
4. Sampling + dilution	Withdraw an aliquot of the filtrate and dilute it to an appropriate concentration for analysis (to be determined in preliminary studies).
5. pH measurement	Measure the pH of the buffer to detect any changes to the pH value during the dissolution process.
6. HPLC analysis	Quantify the concentration of dissolved API via a validated stability-indicating HPLC method.
7. Solubility classify-cation based on the BCS	Calculate mean solubility values.
	Calculate mean dose/solubility ratios with the highest dose strength, the highest single therapeutic dose and the highest dose strength listed on the current EML.
	Classify APIs with one or more ratios larger than 250 mL as “not highly soluble”, APIs with values equal or less than 250 mL at every tested pH as “highly soluble”.

The study protocol presented in Table 1 for solubility determination is based on the shake-flask method. To minimize the amount of necessary material and lower the costs, the experiments can be performed in a miniaturized approach, specifically in Uniprep™ syringeless filter systems, for example with a capacity of three milliliters and a PTFE filtration membrane with a pore size of 4.5 µm. The construction of those filter vials is shown in figure 3. For the experiments for proguanil, cephalexin, and the EML compounds, the original test volume of 250 ml was scaled down to three ml. To calculate the necessary amount of API powder, the minimum solubility value which must be achieved for a classification as “highly soluble” was calculated by dividing the highest dose (highest dose strength or highest single therapeutic dose) by 250 ml. The result was multiplied by three for the use of a three ml Uniprep™ vial. A slight excess to this resulting amount was weighed into the vials. The buffers for the solubility experiments can be chosen according to Table 2. If incompatibilities of the API with buffer components are known, alternative buffer solutions can be selected from the European

pharmacopoeia. All buffers should be prepared according to the instructions of the respective pharmacopoeia.

Table 2. Buffer selection for different pH values according to the biowaiver guidelines

Test pH	**Recommendations by biowaiver guidelines**		**Comments**
	USP[59]	**Int. Ph.[60]**	
1.0	Not available	Not available	USP hydrochloric acid buffer pH 1.2 adjusted to 1.0
1.2	Hydrochloric acid buffer	Not available	Int. Ph. Dissolution buffer pH 1.3 could be adjusted to pH 1.2;[61] Ph. Eur. Dissolution buffer[62] is also feasible
4.5	Acetate buffer	Dissolution buffer, pH 4.5, TS (phosphate buffer)	-
6.8	Phosphate buffer	Dissolution buffer, pH 6.8, TS (phosphate buffer)	-
pK_a	Hydrochloric acid buffer, acid phthalate buffer, neutralized phthalate buffer, phosphate buffer, alkaline borate buffer, acetate buffer and citrate buffer are feasible	Dissolution buffer pH 1.3, 2.5, and 3.5 are feasible	Ph. Eur. buffers[63] are also feasible
$pK_a - 1$			
$pK_a + 1$			

The buffer pH must be checked prior to the experiment and noted for the report. The acceptable deviation for the pH value is extracted from the requirements for the preparation of buffers for dissolution tests of solid oral dosage forms. Since

this study protocol does not include a pH adjustment after addition to the API powder, it is important that the pH value is as exact as possible. The initial pH is compared with the pH after the experiment to provide information about the acidic/basic behavior of the API. Once the media are prepared, samples for every pH and replicate can be set up. The buffer is added to the API powder in the vial and the vial chamber is sealed temporarily. As quickly as possible, the prepared Uniprep™ vial should be incubated and shaken. During the 24-hour incubation time, the status of the solution should be checked occasionally. Especially if the powder dissolves immediately after addition of the buffer, crystals in the solution might indicate a precipitation reaction which should be noted and reported. Changes in color and/or odor are also important to note, as they usually indicate degradation reactions. Any observations regarding these instabilities should be noted and reported with the results of the solubility studies. This applies particularly after the 24 h period before filtration. pH measurements during the study should be avoided to prevent alterations in the sample composition.

After filtration with the Uniprep™ plunger, samples are withdrawn from the filtrate. They are diluted and analyzed promptly to avoid issues regarding the sample solution, e.g. precipitation during cooling. After sample withdrawal and dilution, the pH of the buffer in every vial must be checked (it should not be measured before the sampling since the handling of pH electrodes might adulterate the composition and concentration of the sample solution). The pH of the samples will be measured at room temperature, which is why the pH of the buffer should be adjusted at the same temperature during media preparation.

The determined concentrations of dissolved API should be expressed in mg/ml. Very small numbers can be expressed decimally, for instance ten to the power of minus three (10^{-3}).[55] The solubility of the API at each pH value should be reported as the mean value of all replicates. With these results, the calculations for the different D/S ratios can be made. The chosen dose value should be divided by the solubility value obtained from each individual replicate of the experiment. The calculations are carried out for every pH that was evaluated. For every pH condition, the mean D/S ratio should be calculated and reported. The same procedure applies for each of the doses to be considered (FDA, EMA, WHO) if these differ.

The dose/solubility profile over the tested pH range of every dose should be considered for the solubility classification. Highly soluble APIs are those which show D/S values equal or less than 250 ml at all tested pH values. All others must be classified as “not highly soluble”.

If a “not highly soluble” solubility value is accompanied by a significant change of the visual appearance of the test solution and/or if the analysis of the diluted samples indicates degradation, e.g. by additional chromatographic peaks to the API peak or an altered shape of the API peak, further considerations and potentially additional experiments may be required. Since the information about appropriate evaluation of the solubility of degrading APIs are sparse in the biowaiver documents published by the health authorities, the decision tree published with the solubility classifications of the newly added APIs of the EML can be used as a guide when dealing with degradation challenges.[55] A more specific study protocol for those situations can be found in Table 3.

Table 3. Study protocol for APIs that show degradation in the regular solubility study

Conditions	**Comments**
1. Sample preparation for degradation study, i.e. a stability-indicating solubility study	Evaluate every pH condition at which degradation was observed in a different experiment.
	Prepare buffer solution(s) according to the regular study protocol and prepare a clear solution with the API based on the results of the 24 h solubility experiment or literature values. Filter the solution through a membrane equal to the Uniprep™ filter membrane into a sealable container (e.g. small glass jar).
	Take the first sample right before incubation, dilute and analyze immediately.
2. Degradation test period	Incubation and shaking of the container according to the regular study protocol.
	Withdraw continuously samples; dilute and analyze them immediately. In the first hour, chose small

Conditions	Comments
	sampling intervals, which correspond to the run time and number of injections of the HPLC analysis method for prompt quantification (e.g. chromatographic run time: 7 min, injections: 2 → sampling interval: 15 min). Increase the intervals after the first hour (e.g. sampling every half or full hour).
	Check for potential changes in the appearance of the samples, e.g. a change in color or odor, and note them for the report.
	The degradation study should be carried out for at least 1-3 hours, depending on the pH of the medium.
3. pH measurement	Measure the pH of the solution at the end of the study to detect any changes of the pH value due to potential degradation occurred during the incubation period.
4. Determination of degradation time points	Evaluate the extent of degradation on basis of the concentrations in the withdrawn samples. Express the results in percent of the concentration of the pre-incubation sample. For experiments at pH 1.0 or 1.2 determine whether more than 15% were degraded in 1 hour and for experiments at all other pH conditions whether more than 15% were degraded in 3 hours. If so, note the time point at which 15% of the API had decomposed.
5. Supplementary solubility study	Perform additional solubility studies for those pH conditions at which degradation was observed. Sample preparation can be performed as described in the regular study protocol; incubation and shaking can be started accordingly
	The duration of the incubation period depends on the pH: under gastric conditions, i.e. at pH 1.0 and

Conditions	Comments
	1.2, it should not exceed 1 hour or the 15% degradation time point, while at all other pH values, the incubation period should not exceed 3 hours or the 15% degradation time point.
	Filtration, pH measurement and analysis of the samples can be performed according to the regular study protocol.
6. Solubility classify-cation based on the BCS	Calculate mean solubility values and mean D/S ratios according to the regular study protocol. Classify APIs with one or more ratios larger than 250 mL as “not highly soluble”, APIs with values equal or less than 250 mL at every tested pH as “highly soluble”.

Degradation of a dissolved API is mainly a problem for soluble APIs. It can reduce the concentration of the API in solution and, as a consequence, lead to a false solubility classification. This process might also change the permeability classification, as discussed for the solubility studies with EML compounds.[55] A degradation study is recommended for all pH conditions of the 24 h solubility determination at which decomposition reactions are observed. By only evaluating the degradation at those pH values, time, material and therefore costs can be saved. A single determination without replicates (i.e. n = 1) might also be considered if the API is very expensive. Regardless of the number of replicates, the extent and rate of the degradation reactions can be estimated and thereby the time frame for an additional solubility study can be set. A reasonable amount of API that will dissolve completely in a low volume of buffer, e.g. 5 ml, should be chosen for the degradation study. If possible, the amount should be sufficient to fulfill the “highly soluble” criterion. Literature solubility data or the results from the 24 h experiment can be helpful for these considerations. The clear solution should be filtered through a membrane filter to eliminate undissolved particles and a first sample should be withdrawn before incubation. In this way, all concentrations that are quantified later can be expressed as percentage of the original concentration of the filtrate. During the incubation period, frequently withdrawn samples help to evaluate the degradation process. If possible, the samples should be analyzed

immediately to minimize further instability reactions (although ideally the stability of the API is established with the validation of the method). The appearance of the solution and potential changes of it that were observed during the 24 h solubility study, e.g. precipitation, change in color or odor, should be monitored carefully. The FDA recommends determinations in gastric fluid for one hour and in intestinal fluid for three hours in cases of instabilities in the GI tract,[10] reflecting the usual residence time of an orally administered API in the respective GI compartment in the fasted state.[55] Referring to this suggestion, determinations at pH 1.0 or 1.2 or other pH values which represent gastric conditions should require a minimum incubation period of one hour, all others a period of three hours. The media for the degradation study should be the same as for the regular studies since the enzymes that are present in the gastric and intestinal Simulated Fluids might lead to different solubility results. Although the recommendation of the FDA guidance should be the basis for the duration of additional solubility studies, the results of the degradation study are a further important consideration. Supplementary solubility studies should be performed according to the regular study protocol but for a shorter period which ensures that not more than 15% of the API are degraded.[55] The BCS solubility classification should be made using the results of these supplementary solubility experiments.

The biowaiver monograph should include a detailed report regarding all conditions, measurement, observations, analysis results and additional studies. With these information, it can be discussed whether all scientific and juridical prerequisites for a conclusive solubility classification are fulfilled, whether the API is “highly soluble” or not, and which challenges in terms of stability are worth considering for a biowaiver application.

2.3. The "minimum solubility" approach

As discussed in the previous sections, the guidance documents of the FDA and the WHO require the determination of the equilibrium solubility of an API i.e. the thermodynamic solubility. The thermodynamic solubility is a concrete, comparable value for every substance in a certain solvent at a certain temperature and is the most appropriate choice for the calculations for the solubility classification according to the BCS. However, the determination of the equilibrium solubility has some disadvantages for very soluble APIs. Because of the high amount of substance that might be necessary to determine the equilibrium, a thermodynamic solubility study can be extremely expensive. Even in a scaled-down experimental approach, it might be necessary to use several hundred milligrams per sample and with the replicates at different pH conditions several grams could be necessary to complete the determination.[55] Apart from the costs, the use of several grams of substance just for solubility studies is wasteful and ecologically not sustainable. In addition, the use of high amounts of APIs with acidic or basic properties could lead to the buffer capacity of the medium being overwhelmed in some cases. That would require adjustments of the pH, which is less than ideal for large scale studies but not practical for small-scale studies.

Hence, in the biowaiver monographs of proguanil hydrochloride and cephalexin monohydrate as well as in the publication comprising the solubility classification of several APIs on the EML the approach taken was to determine the "minimum solubility".[30, 31, 55] In the draft protocol for equilibrium solubility studies that was published by the World Health Organization in 2018, and which complements the WHO biowaiver guideline, exceptions from the principle to evaluate the equilibrium solubility are specified: if the required amount of API cannot be provided and/or is unaffordable or if the buffer capacity of the pharmacopoeial buffers would be exceeded (as may be expected if the API is very soluble, as discussed above).[14] In those cases, experiments with the highest therapeutic single dose gathered from the summary of product characteristics in a volume of 250 mL (or proportionally smaller set-ups) are justified. The "minimum solubility" approach uses the minimum amount of an API, which – if completely dissolved in the respective volume - results in a D/S ratio of 250 ml or less. The dose plus a small

excess (usually 10%) is added to 250 ml of buffer solution and the concentration of API is determined after 24 hours at 37°C. Scaled-down experiments are also reasonable. If the analysis shows that all API has been dissolved, the concentration represents the “minimum solubility” (expected for very soluble APIs). This value can be used for the calculation of the D/S ratio. Since the solubility is thereby reported as a minimum value, the calculated D/S ratio will be a maximum value. As long as the D/S ratio is below 250 ml for all media, the drug can be classified as “highly soluble”. Table 4 compares the main characteristics of the solubility determination method according to the FDA and WHO biowaiver documents with the “minimum solubility” approach.

Table 4. Comparison of main characteristics of solubility studies evaluating the equilibrium solubility according to the FDA and WHO biowaiver guideline or the "minimum solubility"

Characteristics	**Equilibrium solubility**	**“Minimum solubility”**
API	Thermodynamic solubility for APIs with doses similar to or greater than the amount likely to be soluble in 250 ml	“Minimum solubility” for APIs where the dose is far lower than the amount likely to be soluble in 250 ml / thermodynamic solubility in 24 hours for APIs with similar or greater doses
Preliminary tests	Estimation of necessary amount of API, necessary equilibrium time and potential pH adjustment	No preliminary tests necessary
Required amount of API	Small excess to the amount in mg that is estimated to be soluble in a volume of 250 ml	Dose in mg plus a small excess (scale down if a small-volume method is used)
Duration	Until achievement of equilibrium (up to 72 h)	24 hours

Characteristics	Equilibrium solubility	"Minimum solubility"
Eligibility for scale-down	Yes	Yes
calculation for solubility classification	D/S ratio is calculated with the dose divided by equilibrium solubility	Maximum D/S ratios calculated with dose divided by "minimum solubility"

2.4. Precipitation – case example proguanil hydrochloride

An important step during a solubility study for a biowaiver monograph is to check the dissolution status at the end of the incubation period. It is possible to visually detect a residue at the bottom of the Uniprep™ vial. A residue may be present for different reasons. Optimally, it is the physicochemical unchanged excess of the API powder that was weighed in the vial, indicating that the solution is saturated and the equilibrium is reached. Another possibility is that the residual substance consists of the API which precipitated in another polymorphic form than the form which was weighed in for the experiment. It might also originate from a decomposition reaction of the API resulting in degradation products which are not soluble. In another case, the formed precipitate consists of a complex or a salt built from the API and buffer ions, which lowers the solubility product.

Precipitation, most probably caused by a complex or salt reaction, was observed after the first and also after the final solubility studies for proguanil hydrochloride at some pH conditions.[30] The tendency of proguanil hydrochloride to react with buffer media was already observed during pilot studies conducted to choose appropriate buffers. The pK_a of proguanil hydrochloride that is relevant for the biowaiver solubility studies is 2.3. Therefore, solubility studies at a pH of 2.3 and at one pH unit below (1.3) and above (3.3) the pK_a, respectively, were necessary for a guideline-conform pH-solubility profile in addition to the evaluations at pH 1.0/1.2 (hydrochloric acid buffer), 4.5 (acetate buffer), and 6.8 (phosphate buffer). Following the study protocol shown in Tables 1 and 2, buffers at the extra pH values were chosen from the list of buffer solutions of the USP.[59] For pH 1.3, a hydrochloric acid buffer was selected. For pH 2.3 and 3.3, the USP provides an acid phthalate buffer. Small-scale tests with proguanil and the phthalate buffer revealed an immediate flocculation, likely due to salt formation. In order to find another appropriate medium, (compendial) citrate and maleate buffers with pH 2.3 and 3.3 were tested, but these showed similar reactions. It was concluded that phosphate ions from a phosphate buffer might lead to comparable problems (it was postulated that the flocculation would also occur at pH 6.8, since most buffers at this pH are phosphate buffers and both, the USP and the Int. Ph., recommend the use of a phosphate buffer at this pH).[59, 61] For this reason, phosphate

buffers pH 2.3 and 3.3 with a phosphate concentration as low as possible were chosen for the study (European Pharmacopoeia).[63] The addition of the buffer to the powder showed no immediate reaction and proguanil seemed to dissolve completely (also in the phosphate buffer at pH 6.8). However, after the 24-hour incubation interval, residues were visible in all vials that contained a phosphate buffer. As shown in Table 5, the solubility values determined under these conditions were significantly lower than the values at other pH points, for example at pH 1.2 and 4.5. The lowest value was observed at the pK_a. The solubility at pH 6.8 was similar to the solubility determined at pH 3.3, which was an unexpected result. However, observations confirmed the hypothesis that proguanil hydrochloride would also interact with phosphate ions. The phosphate buffer solution R1 from the European Pharmacopeia contains the highest amount of phosphate ions of all buffers used for the solubility studies.[63] Therefore, the low solubility value obtained in that medium is in line with formation of a poorly soluble salt with phosphate. Alternative compendial buffers without any critical counter ions were tested for the three pH conditions, e.g. USP hydrochloric acid buffer pH 2.3,[59] Ph. Eur. buffer solution pH 3.5 (acetate-hydrochloric acid),[63] DAB 7 acetate-borate buffer pH 6.85,[64] and Ph. Eur. 1 M tris-hydrochloride buffer solution pH 6.8.[63] The results of the solubility experiments in the alternative buffers are also shown in Table 5.

Table 5. (Minimum) solubility results with percentage dissolved of the test amount of proguanil hydrochloride in different pharmacopoeial buffers over a pH range of 1-6.8

pH condition	**Original Study**	**Alternative**	**Comments**
	• **Buffer** • **Determined solubility in mg/ml (Mean ± SD)** • **Amount dissolved (Mean ± SD)**		
1.0	Hydrochloric acid buffer pH 1.2 USP, adjusted to 1.0		
	4.3 ± 0.4		
	100%		

pH condition	Original Study	Alternative	Comments
	• **Buffer** • **Determined solubility in mg/ml (Mean ± SD)** • **Amount dissolved (Mean ± SD)**		
1.2	Hydrochloric acid buffer pH 1.2 USP		
	4.21 ± 0.11		
	100%		
1.3	Hydrochloric acid buffer pH 1.3 USP		
	4.1 ± 0.9		
	100%		
2.3	Buffer solution pH 2.2 Ph. Eur. (phosphate buffer), adjusted to 2.3	Hydrochloric acid buffer pH 2.3 USP	Crystalline residue after incubation period of original study, alternative buffer medium avoids precipitation, buffer capacity not ideal[30]
	1.35 ± 0.29	5.3 ± 0.4	
	32.66%	100%	
3.3	Phosphate buffer solution pH 3.2 Ph. Eur., adjusted to 3.3		Crystalline residue after incubation period of original study, no appropriate alternative pharmacopoeial buffers found – final solubility result
	2.58 ± 0.26		
	60.27%		

pH condition	Original Study	Alternative	Comments
	• **Buffer** • **Determined solubility in mg/ml (Mean ± SD)** • **Amount dissolved (Mean ± SD)**		
4.5	Acetate buffer solution pH 4.5 Ph. Eur.		
	4.7 ± 0.6		
	100%		
6.8	Phosphate buffer pH 6.8 USP	Phosphate buffer solution pH 6.8 R1 Ph. Eur.	Large crystalline residue after incubation period of original study, buffer with higher phosphate concentration decreases solubility value
	2.89 ± 0.06	0.94 ± 0.09	
	74.03%	21.75%	

The change of the buffer medium for the solubility determination at pH 2.3 from a phosphate to a hydrochloric acid buffer resulted in a clear solution without precipitates and increased the solubility value from 1.53 to 5.3 mg/ml. In the latter case, a minimum solubility was determined since 100% of the weighed-in powder was dissolved. Instead of the notation as a mean value with the standard deviation, the “minimum solubility” is presented as greater than or equal to value (≥ 5.3 mg/ml) and the results of the EML solubility studies were published in this way.[55] Although, it must be noted, that the change in the pH from 2.3 to 2.8 which was observed at the final pH measurement indicates that the buffer capacity was exceeded.[30] Since the ability of the USP hydrochloric acid to control the pH is lower at higher pH values, this result was expected. Nevertheless, other buffers should be selected at this pH for APIs with stronger basic properties. For the determination at pH 3.3, no appropriate non-phosphate buffer could be found. Therefore, the result from the study in the phosphate buffer was published with the final solubility results and an additional information of the final dissolution status. A

“minimum solubility” value could not be reported at pH 6.8 either. As observed for pH 2.3 and 3.3, the proguanil hydrochloride powder dissolved immediately after the addition of the buffer. After the incubation, a crystalline residue could be detected in the pH 6.8 vials and only 74% was still in solution. Thus, it can be assumed, that the missing 26% were dissolved initially and precipitated later with the phosphate ions, but only to a minor degree compared to the experiment with the phosphate buffer solution R1 from the Ph. Eur.. The change of the test medium to a Ph. Eur. buffer solution decreased the amount of dissolved proguanil hydrochloride from ~ 74% to ~22% and was accompanied by a sediment consisting of large crystals. The difference between the phosphate concentration in these two buffer solutions explains the difference in results at pH 6.8.

The results at all three critical pH values nevertheless fulfill the “highly soluble” criterion of the BCS, with maximum D/S ratios of $\leq$ 250 in each case ml.[30] Only if the highest therapeutic single dose were to change to more than 400 mg, would the BCS classification of proguanil hydrochloride.

The proguanil case example illustrates why experimental conditions that influence the results of the solubility determination should be avoided. False negative decisions as to the eligibility for a biowaiver must be avoided and therefore, physicochemical incompatibilities should be checked in advance and, if possible, excluded by choosing appropriate experimental conditions. In case of an unavoidable influence of the experimental conditions, as in the case of the proguanil solubility determination at pH 3.3, all information regarding inconclusive results must be reported.

The problems encountered with the phosphate buffer lead to an obvious disadvantage of the current methodology for the solubility determination over a physiological pH range. Although phosphate buffers are often used for studies in the context of a BCS classification (e.g. pharmacopoeial buffers,[59, 61, 63] Simulated Intestinal Fluid (SIF),[65] Fasted State Simulated Intestinal Fluid (FaSSIF)[66]), they do not reflect the physiological conditions in the gastrointestinal tract perfectly: the buffer capacity is too high and bicarbonate, not phosphate, is the main buffer in the fasted intestinal fluids. The use of a maleic acid-sodium chloride-sodium

hydroxide buffer, which is the basis for the composition of FaSSIF-V2 (FaSSIF Version 2),[67] a biorelevant medium, is an even worse choice in the case of proguanil. The ideal buffers for the solubility determination would be those similar to the human fluids at the respective pH, e.g. a bicarbonate buffer for the conditions in the upper small intestine (pH 6.8).[68] Since this is a condition that is not practicable due to the instability of the pH in bicarbonate buffer, it is at advisable to choose a buffer species which doesn't interact negatively with the drug under study. Biorelevant media come closer to the *in vivo* situation in terms of buffer capacity, but to date are not mentioned in the guidances. These media have a composition closer to that of intestinal fluids, containing for example bile salts which can enhance the solubility of the API and therefore lead to lower dose/solubility ratios. The only real benefit of the conservative approach currently taken, which is based on the use of compendial buffers with not bile components, is the lower risk of false positive biowaiver decisions.

In the case that the test conditions cannot be changed to avoid their influence on the solubility results, a suitable approach to evaluate the precipitation characteristics of an API might be a precipitation study which observes the progress of the precipitation at frequent intervals. Furthermore, solid-state characterization of the precipitate would provide insight to any morphology or chemical changes that the API has undergone.

2.5. Degradation – case example cephalexin monohydrate

Physicochemical instabilities include degradation reactions caused by light, temperature, pH, air humidity etc. In the case of cephalexin monohydrate, a pH-dependent instability in aqueous solutions has been extensively reported in the literature.[69–72] In general, the development of stability-indicating methods for study and analysis of cephalexin were quite complex. For example, cephalexin can be purchased as a "hydrate" with the molecular formula $C_{16}H_{17}N_3O_4S \cdot xH_2O$ (x not specified) and the CAS number 1820673-23-1.[73] It is clearly different to cephalexin monohydrate with the formula $C_{16}H_{17}N_3O_4S \cdot H_2O$ and the CAS number 23325-78-2.[73] The difference is important, since the anhydrous form and the dihydrate are also registered substances which show different physicochemical characteristics, e.g. a different solubility or hygroscopicity.[74–76] In addition, the solubility of cephalexin monohydrate in organic solvents like acetonitrile or methanol seemed to be low. The preparation of standard solutions of cephalexin monohydrate in organic solvents for the validation of the analysis method and calibration before the quantification was not possible, e.g. linearity in methanol could not be established: the results were not reproducible. The poor solubility in organic solvents was also the reason for the choice of the phthalate buffer at pH 3.7, since the buffer solution pH 3.7 of the European Pharmacopoeia contains ethanol and was therefore not suitable for the studies with cephalexin.[63] Table 6 shows the results of the 24-hour solubility study at all relevant pH conditions including the percentage that was dissolved from the amount weighed into the vials and including observations that were made after the incubation.

Table 6. (Minimum) solubility results with percentage dissolved of the test amount of cephalexin monohydrate in different compendial buffers over a pH range of 1-6.8

pH condition	**Buffer/ (Minimum) Solubility in mg/dl/ Percentage dissolved**	**Comments**
1.0	Hydrochloric acid buffer pH 1.2 USP, adjusted to 1.0	Sulfurous odor, bubbles, determination of "minimum solubility"
	≥ 4.15	
	100%	
1.2	Hydrochloric acid buffer pH 1.2 USP	Bubbles, no visible powder residue
	4.09 ± 0.33	
	98.41%	
1.7	Hydrochloric acid buffer pH 1.7 USP	Sulfurous odor, bubbles, no visible powder residue
	4.36 ± 0.3	
	94.73%	
2.7	Phosphate buffer solution pH 2.8 Ph. Eur., adjusted to pH 2.7	Bubbles, no visible powder residue
	4.65 ± 0.52	
	96.96%	
3.7	Phthalate buffer solution pH 3.6 Ph. Eur., adjusted to pH 3.7	Sulfurous odor, small bubbles, slightly yellow color
	3.90 + 0.46	
	89.12%	
4.5	Acetate buffer solution pH 4.5 Ph. Eur.	Strong sulfurous odor, deep yellow color
	2.57 ± 0.28	
	55.71%	

pH condition	Buffer/ (Minimum) Solubility in mg/dl/ Percentage dissolved	Comments
6.8	Phosphate buffer pH 6.8 USP	Yellow color
	2.38 ± 0.08	
	56.79%	

After incubation, the appearance of the initially colorless solution had changed at all pH conditions. Most of the samples showed numerous bubbles at the wall of the vials. They might be the gaseous product of a (degradation) reaction during the incubation period but could also have consisted of air which had been solubilized at room temperature before the incubation. A sulfurous odor could be detected from some samples but could not be assigned to all vials with bubbles. In comparison to the samples with lower pH values, residues of powder could be observed in the vials with the pH conditions of 3.7, 4.5 and 6.8. In addition, the color of the solution had changed to yellow at these three conditions. The darkest color was observed at pH 4.5. Especially the change in the color indicated a chemical reaction during the incubation period. A reaction, in fact a degradation of cephalexin is well described in the literature under pH conditions of 6.8 or above.[70, 72] Therefore, a low solubility value at this pH was expected. By contrast, the literature reports the highest stability for cephalexin at pH 4.5.[71] Hence, the similar (or an even more intense) reaction observed at this pH was completely unexpected. However, additional peaks in the chromatograms of the samples at both pH values supported the assumption of a degradation reaction. The resulting solubility at pH 3.7 (mean value 3.90 ± 0.46 mg/ml) would result in a D/S ratio of 259 ± 29 ml. Regarding the BCS cut-off value, this result would clearly lead to a borderline decision as to the BCS class. Since the solution color had changed and powder residues were visible in the pH 3.7 vials, the pH was taken into consideration in the ensuing degradation study according to the study protocol (see Table 3). Single cephalexin samples were tested at each of the three pH conditions and analyzed immediately to ensure the stability of cephalexin monohydrate. Because of a runtime of 10 mins for the HPLC analysis and the minimum of two repeats, the sampling time for the first hour was every 20 minutes. Table

7 shows the percentage of quantified cephalexin monohydrate at each time point relative to the quantified amount in the first sample which was withdrawn immediately before the incubation.

Table 7. Percentage of quantified cephalexin monohydrate for the period of the degradation studies performed at pH 3.7, 4.5 and 6.8

Sampling time point [min]	**Percentage of quantified cephalexin monohydrate in related to the quantified amount at 0 mins**		
	pH 3.7	**pH 4.5**	**pH 6.8**
0	100	100	100
20	100	100	99
40	101	99	99
60	105	97	99
120	99	93	94
180	101	96	93
240	101	96	90

It must be noted that these are values obtained from a study design with n=1. For determination of the degradation rate, a higher number of samples would be needed. However, the study described above was only intended to serve as a rough estimate for the decision on the duration of the subsequent solubility study. Ideally, a duration that is relevant to the physiological process of intake, dissolution and absorption of an immediate release solid oral dosage form can be chosen. Based on the results of Table 7, the incubation time for the supplementary solubility study was set to three hours. An abbreviated solubility study was not performed at pH 3.7 since the dissolved amount did not decrease during the four-hour degradation study. A potential alternative explanation for the low solubility obtained in the 24-hour study is poor wettability of the powder. The results of the supplementary solubility studies at the two other pH values are shown in Table 8.

Table 8. Results of supplementary solubility studies of cephalexin including the percentage dissolved of the test amount at pH 4.5 and 6.8

pH condition	**(Minimum) Solubility 3 h in mg/ml**	**Percentage dissolved**	**(Maximum) D/S ratio 3 h in ml (1000 mg)**
4.5	4.13 ± 0.71	95.61%	247 ± 47
6.8	4.14 ± 0.31	88.50%	243 ± 18

Table 8 shows an obvious increase of more than 50% for the solubility of cephalexin at pH 4.5 and 6.8 obtained from the three-hour solubility study compared to the values of the regular 24 h study. The corresponding HPLC chromatograms from the quantitative analysis did not show any critical characteristics. Both findings corroborate a theory of a time-dependent degradation reaction of cephalexin in buffer solutions of 4.5 and 6.8. However, the values for the D/S ratio calculated with a dose of 1000 mg cephalexin indicate a borderline decision in terms of the BCS class. The mean values are below 250 ml, but taking the standard deviations into account, the values might exceed the BCS class cut-off value. In addition, the percentage dissolved values are below 100%. At pH 6.8, no powder residues were visible at the end of the three-hour incubation period. Therefore, the lower percentage does probably not originate from a reduced dissolution, but the beginning of the degradation reaction which is in accordance with the results from the degradation study. At pH 4.5, the vials showed a few powder grains at the bottom of the test chamber. Possible explanations for this observation include an insufficient shaking rate during incubation and/or wettability problems. Once more, the color of the cephalexin/pH 4.5 buffer solution had turned (light yellow), although a significantly higher amount of cephalexin was quantified. This was also observed during the degradation study at pH 4.5. Therefore, a further root cause analysis evaluating the correlation of the color change with the degradation reaction (e.g. degradation product with high extinction coefficient in the yellow range) would be interesting. Additionally, the influence of the pH and buffer components on the phenomenon should be investigated, given, that cephalexin was stable in the mobile phase which consisted mainly of water with a measured pH of 5 for more than 24 hours and considering literature reports that attest that the highest stability of cephalexin is at pH 4.5.

A decision tree has been published for solubility determinations in the context of BCS-based biowaivers, taking into account degradation.[55] Besides APIs which show no degradation in the 24-hour solubility study and can therefore be classified using the results of the regular solubility determinations, there are APIs which show degradation at all or certain pH conditions of the original study. In the case of cephalexin, the solubility in acidic i.e. gastric media is high. Hence, for the solubility classification and also the absorption process, the amount of cephalexin that is available despite the degradation process in intestinal media must be determined. In such cases, a shorter, supplementary solubility whose results are included in the BCS classification is performed. For cephalexin the three-hour study results at pH 3.7, 4.5 and 6.8 as well as the 24-hour study results were used to define the solubility characteristics. Although the results in the three-hour studies could be considered borderline because of the standard deviations, cephalexin monohydrate was classified as “highly soluble” using the mean values. Because the degradation at pH 4.5 and 6.8 did not exceed 15% in three hours, the permeability classification was not affected.[55]

For other drugs, it is not necessary to know the solubility after a physiologically relevant time period, for example if the solubility at pH conditions reflecting the absorption site (pH 6.8) does not meet the “highly soluble” criterion and thus the assignment to BCS Class I or III is not possible. Folic acid and rifabutin, which show degradation in acidic media, are examples of this type of substance. Nevertheless, the degradation behavior for APIs like these should be reported and discussed.

2.6. Optimization of the "minimum solubility" determination approach

The study protocol for the "minimum solubility" approach was developed for solubility studies in the context of BCS-based biowaivers and takes the requirements of the biowaiver guidelines published by health authorities FDA, WHO and EMA into consideration. Therefore, results which are obtained by solubility studies with this design could be used for a biowaiver procedure. It can be used not only for very soluble APIs but also for those which have less favorable D/S ratios, since in those cases the attempt to dissolve the dose in 250 ml of buffer (or the equivalent in the scaled down approach) will also permit a decision about whether the API is highly soluble or not. Thus, the "minimum solubility" determination approach allows a fast, in some cases even a visual distinction whether the cut-off criterion for the solubility classification according to the BCS is fulfilled or not.

The ability to screen a high number of substances, e.g. a screening of APIs that are listed on the WHO EML and whose qualification for a biowaiver procedure is a matter of particular interest, can be realized with the "minimum solubility" approach.[55] It could be followed by other screening studies, especially since the amount of required materials, e.g. API, buffer and filter, and as a result the material costs are comparably low. Since preliminary studies to determine the equilibrium time are not necessary, the time that must be invested for the study is limited. Indeed, the most time-consuming step is the development and validation of the HPLC analysis method. In many cases, the use of a pharmacopoeial method including a system suitability test can reduce the time to establish an analysis method. In other cases, a simple reversed phase chromatography method with a two-component mobile phase (e.g. acetonitrile/water with the opportunity to adjust the pH according to the properties of the tested API) is sufficient. Despite a potential time investment and costs associated with this quantification method, the HPLC analysis is recommended in order to obtain information about potential physicochemical instabilities occurring during the solubility study.

An ideal solubility determination for a biowaiver monograph, which disregards the factors costs and time, should consider some additional aspects. Steps both before and after the incubation have potential for improvement or adding investigations. The media which are required for solubility studies by the health authorities and which are used for the presented approach reflect only the pH conditions of the different aqueous media to which an API in an immediate release solid oral dosage form is exposed during the passage through the gastrointestinal tract. Other characteristics, including the presence of surfactants and enzymes in gastrointestinal fluids, are neglected when using compendial buffers. The incorporation of such components should lead to solubility results with a higher physiological relevance. Although the authors of the original Biopharmaceutics Classification System did not define particular physiologically relevant media as basis for the classification, they emphasized the importance of an *in vitro* setting reflecting the *in vivo* conditions.[2] Since various simulated gastric and intestinal media on basis of buffers have become available since then, solubility studies in these biorelevant media, e.g. fasted state simulated gastric or intestinal fluid (FaSSGF or FaSSIF), could be a valid addition.

Not only the choice of medium but also the preparation of the medium is worth reconsidering. Different pharmacopoeias require a degassing procedure for buffers that are used for dissolution tests.[62, 77] In the light of the results of the cephalexin solubility study, that requirement should also apply for media used for solubility studies. Numerous gas bubbles were observed in the cephalexin test tubes after the incubation, some of which were attached to the cephalexin powder particles and reduced the wettability of the solid API. In cases where bubble formation is not due to the gaseous product of a chemical reaction between API and medium, degassing the test medium would reduce or exclude the impact of dissolved air in the aqueous media on the solubility results.

In cases where a residue is visible during or after the incubation period, different reasons for its origin are conceivable. The solid particles might look unchanged compared to the powder particles that were weighed in the test vial indicating wettability issues or a poor solubility of the tested API. But if the solid state looks different to the initial texture of the particles, it might be a solid polymorphic form

of the API or a solid product from a reaction of the API with the medium that precipitated during the incubation. In the latter cases, a characterization of the nature of the residue would provide essential information about necessary changes in the study design, e.g. the choice of test medium for more conclusive solubility results.

After incubation, all undissolved particles should be separated from the solution by filtration. The syringeless filters which were used in the solubility protocol described herein were chosen because of the convenient handling and the low material costs. The material of the filter membrane, polytetrafluorethylene (PTFE), is also utilized in conventional shake-flask solubility studies, in which the filtration is carried out with a syringe and a filter. Filters consisting of other membrane or fiber materials, e.g. nylon, polyvinylidene fluoride (PVDF) or glass fiber, are available. PTFE is a particularly inert material and should therefore not influence the solubility results by API/filter reactions. It has also been shown to have lower adsorption properties than many other filter materials.[78] Nevertheless, depending on the characteristics of the tested API and the chosen media, specific absorption studies are appropriate, especially if low concentrations in the filtrate are not expected.

In some cases, the solubility value itself requires further consideration, especially for borderline APIs. For proguanil hydrochloride, the maximum dose was divided by the mean solubility value and the result was rounded to a whole number.[30] Since the D/S ratios of all pH conditions are in a similar range and far from the BCS cut-off value of 250 ml, a simple number is sufficient and easy to use for the BCS classification. For cephalexin monohydrate on the other hand, the D/S values are close to the cut-off value.[31] In this case it is recommended to calculate the D/S ratios by dividing the dose by each solubility value obtained per pH and average over the results. Though this calculation leads to the same mean value for the reported D/S ratio at a given pH (e.g. proguanil hydrochloride pH 1.0: ~93 ml vs. ~94 ml, cephalexin monohydrate pH 1.0: ~241 ml vs. ~241 ml), calculating and reporting the mean value plus the standard deviation or even the individual values can make a difference, since it is possible that some values may exceed the 250 ml cut-off. This second way of calculation is also advisable if the solubility

values of each sample differ from each other to a great extent. It is conceivable that some observations or calculations do not allow a conclusive solubility classification, e.g. if the API powder only dissolves completely in two of three samples due to wettability or other, unidentified, problems. In this case, a study setup with more samples than the minimum requirement of n=3 might be helpful since it would reduce the impact of outlier results. Even if this cannot be realized, the standard deviation can be used for a solubility classification considering the worst case.

3. Summary and Outlook

3.1. Summary

The work of this thesis contributed to two different projects. On the one hand, the biowaiver monograph series – a publication series pursued by the FIP focus group “Bioclassification/Biowaivers” - was continued with the publication of the biowaiver monographs for proguanil hydrochloride and cephalexin monohydrate. On the other hand, the determination of the solubility of newly added APIs of the 16th and 17th version of the EML at key pH values realized a continuous BCS classification of essential APIs formulated in immediate release solid oral dosage forms, a project which was initiated by the WHO.

A common interest of both projects is the solubility characterization as first step of the BCS classification of APIs listed on the WHO model list of essential medicines. Only highly soluble APIs, i.e. BCS Class I and III APIs, are eligible for the BCS-based biowaiver. This approval procedure for generic solid oral drug products allows the determination of bioequivalence based on *in vitro* dissolution tests if certain requirements are fulfilled by the API candidate and the product formulation. Waiving time- and cost-intensive pharmacokinetic bioequivalence studies in humans, the BCS-based biowaiver approach enables the approval of generic drug products, which meet high quality standards and can be offered at an affordable prize at the same time. High-quality generic products are an important tool of global public health care since the widespread availability of affordable high-quality medicines is crucial, especially when it comes to essential medicines and the control of diseases for which only a few and/or expensive treatments are available.

Proguanil hydrochloride and cephalexin monohydrate are two of the essential medicines, which are listed on the EML. Proguanil hydrochloride is an antimalarial API, that is mainly used for prophylaxis, and cephalexin monohydrate is an anti-infective agent belonging to the group of cephalosporin antibiotics. The biowaiver monographs of both APIs present solubility values over a pH range of 1-

6.8, which were obtained in experimental solubility studies. The resulting dose/solubility ratios demonstrate that proguanil hydrochloride and cephalexin monohydrate are “highly soluble” according to the BCS biowaiver guidelines of the health authorities FDA, EMA and WHO (proguanil hydrochloride: BCS Class III, cephalexin monohydrate: BCS Class I). Therefore, both APIs are eligible for a BCS-based biowaiver approval.

Biowaiver monographs are published under the auspices of the FIP focus group “Bioclassification/Biowaivers” and present an assessment of all information about an API that are regulatory relevant for a submission to the health authorities and for a potential approval of a generic product of this API via a BCS-based biowaiver: general characteristics with a focus on the therapeutic index and toxicity of the API, its physicochemical und pharmacokinetic properties, existing dosage forms (including excipients) and their performance regarding dissolution and bioequivalence. Where literature information about solubility characteristics and dissolution behavior are inconclusive or not available, the authors can provide data from additionally conducted studies to compete the assessment.

While the biowaiver monographs discuss all prerequisites for a BCS-based biowaiver procedure that must be fulfilled by proguanil and cephalexin as API and as generic product formulation, the publication regarding APIs, which were newly added to the 16th and 17th edition of the EML and which have various indications, presents a screening of these APIs with respect to their eligibility for a biowaiver based on the BCS Class. Nine of 16 APIs, whose solubility was determined at regulatory key pH values (pH 1.2, 4.5 and 6.8) due to insufficient literature data, are “highly soluble” according to the biowaiver guidelines. Based on their BCS class, those APIs are eligible for the biowaiver procedure.

Both projects illustrate that experimental solubility studies are essential in case that research results from the open pharmaceutical literature are incomplete or inconclusive, i.e. solubility values at certain pH values are not available or values from one source are inconsistent with those of another source. In the context of a biowaiver monograph, the design of a solubility study should fulfill all regulatory criteria regarding an appropriate solubility classification of an API but should also

meet the needs of those who aim to provide solubility characterizations of several APIs in order to prepare future biowaiver monographs primarily of those APIs which are “highly soluble” and therefore currently eligible for the BCS-based biowaiver procedure. The solubility determination method presented in this work complies with those requirements, i.e. solubility values obtained with this method could be submitted to the health authorities as part of a BCS biowaiver request. At the same time, the method is suitable to screen several APIs with respect to their BCS solubility class since it allows a fast and cost-effective determination of reliable data. The study protocol that was initially published with the results of the solubility study regarding APIs from the 16th and 17th version of the EML illustrates the concept of the solubility determination method. It is based on a small-scale set-up of the shake-flask method to determine the equilibrium solubility of an API. Furthermore, the method uses the highest dose (highest dose strength or highest single therapeutic dose) of an API as necessary amount for solubility experiments to determine whether the API is “highly soluble” or not according to the definition of the BCS biowaiver guidelines. If the dose plus a slight excess is completely dissolved in a volume of 250 ml of aqueous medium (or – according to the miniaturized set-up – a scaled-down amount of API is dissolved in a corresponding volume), the API is “highly soluble”. The solubility results represent a “minimum solubility” that is expected for very soluble APIs. Details regarding the steps of the study protocol and the concept of the “minimum solubility” approach were provided with this thesis.

Additionally, challenges that might occur during solubility studies, e.g. precipitation or degradation, were addressed. Precipitation reactions were observed for proguanil hydrochloride. Several buffer media that were chosen to represent the required pH conditions in accordance with the biowaiver guidelines showed precipitates directly after the addition of the API or later. Consequentially, the amount of dissolved proguanil was low in these media at the time of analysis. However, it was increased by the choice of other compendial buffer compositions so that proguanil hydrochloride was classified as “highly soluble” as a result. Therefore, this work does not only present a compilation of buffer media, which are recommended by the biowaiver guidelines, but also discusses the use of other suitable buffer media.

Cephalexin monohydrate on the other hand showed degradation at certain pH values during the solubility study. A subsequent degradation study at these pH values determined the extent of degradation and – based on the results – a reasonable time frame for a supplementary solubility study (three hours), which complies with the duration of the relevant physiological processes for a solid oral dosage form (intake, dissolution, and absorption). The results of the additional solubility study together with those of the regular solubility study demonstrated that cephalexin is a highly soluble API according to the BCS biowaiver guidelines. Since the degradation did not exceed 15% in the chosen time frame, the permeability classification (“highly permeable”) was not affected and cephalexin monohydrate was classified as BCS Class I API. A discussion of degradation challenges was initially published with the results of the solubility determinations of the newly added APIs of the 16th and 17th edition of the EML. Following the recommendations of this publication, the study protocol of the present work includes details on the performance of degradation and supplementary solubility studies but also addresses key aspects of a stability-indicating analysis method.

In conclusion, the presented solubility determination method (the “minimum solubility” approach) is an optimized approach for solubility studies in the context of BCS biowaiver monographs. The provided study protocol is applicable for solubility studies whose results – together with other data – should be submitted to the health authorities for a biowaiver application but could also be used to establish solubility characterizations and therefore BCS classes of many APIs in advance to further evaluations. Certain steps of the study protocol can be optimized further, e.g. use of complex buffer media, solid state characterizations, adsorption studies for filter material, etc., which would increase the informative value of the study but at the same time the complexity of the method.

3.2. Outlook

The “minimum solubility” approach has great potential to be applied as a simple, standard method for solubility classifications in the context of BCS-based biowaivers and can be extended in the context of pharmaceutical development.

The protocol for the “minimum solubility” approach could be adopted in the biowaiver guidelines as an alternative way to determine the equilibrium solubility, for example as part of an appendix to the guidelines. Since the determination method is in many ways simpler and cheaper than standard solubility methods, it could be applied worldwide to establish the solubility class of biowaiver candidates.

Continuing the present work, the solubility determination approach should be used for the solubility classification of all APIs of the WHO EML which are listed as immediate release solid oral dosage forms. Listing the BCS solubility classes in a database would provide an overview of all APIs which are eligible for the biowaiver procedure. Only the eligible APIs would be assessed further, for example in a biowaiver monograph, with regard to the other prerequisites for an approval based on *in vitro* dissolution data rather than on pharmacokinetic studies *in vivo*. Thereby, time and costs could be saved while providing wider application of the biowaiver procedure at the same time. This would serve the global aim of making quality medicines available at an affordable price.

The “minimum solubility” approach can also be applied during the pharmaceutical development of a new API for oral administration as soon as a potential dose range is established. For this purpose, the compendial media of the solubility determination method should be replaced by biorelevant media which reflect the conditions in the GI tract better, as has been proposed by Rosenberger et al. in the refined Developability Classifications System.[79] In case that a residue remains after the incubation period, additional solid-state characterizations could help to predict the precipitation behavior of the drug. Evaluating whether the dose is soluble in a volume of 250 ml of physiologically media or not, is a far more useful tool than the equilibrium solubility.

4. German summary

Die vorliegende Arbeit setzt sich aus zwei verschiedenen Projekten zusammen. Die sogenannte „Biowaiver Monograph Series“ ist eine Publikationsserie, deren einzelne Publikationen (Monografien) von einer Arbeitsgruppe der FIP mit dem Themenschwerpunkt „Bioclassification/Biowaiver“ veröffentlich werden. Die Monografien dieser Serie behandeln jeweils einzelne Arzneistoffe, die von der WHO als unentbehrliche Arzneistoffen eingestuft und auf der Liste der unentbehrlichen Medikamente („WHO Model List of Essential Medicines“, kurz „EML“) in festen Darreichungsformen aufgeführt werden. Unentbehrliche Arzneistoffe sollten weltweit jederzeit in qualitativ überprüften Arzneiformen zu einem erschwinglichen Preis zugänglich und in angemessenen Mengen verfügbar sein. Im Rahmen dieser Arbeit wurden Proguanilhydrochlorid und Cefalexinmonohydrat zur Fortsetzung der Reihe ausgewählt. Daneben wurde mit einer Studie zur Löslichkeit von Arzneistoffen, die auf der 16. und 17. Version der EML neu als essenziell eingestuft wurden, ein von der WHO initiiertes Projekt zur Bestimmung der BCS-Klasse essenzieller Arzneistoffe in schnellfreisetzenden, festen, peroralen Darreichungsformen fortgeführt.

Ein erster Schritt und damit ein gemeinsames Interesse bei beiden Projekten war die Bestimmung der Löslichkeit für die BCS-Klassifizierung von Arzneistoffen der Model List of Essential Medicines der WHO. Aktuell sind gemäß den Richtlinien der Gesundheits- und Zulassungsbehörden FDA, EMA und WHO nur hochlösliche Arzneistoffe (BCS Klasse I und III) für eine Zulassung per BCS-Biowaiver Verfahren geeignet. Dieses spezielle Zulassungsverfahren für generische Arzneimittel erlaubt die Bestimmung der Bioäquivalenz mithilfe von *in vitro*-Freisetzungsuntersuchungen anstatt von pharmakokinetischen oder anderen *in vivo*-Studien, vorausgesetzt, dass der Arzneistoff und die Formulierung der Darreichungsform jeweils bestimmte Anforderungen erfüllen. Das BCS-Biowaiver Verfahren erleichtert die Marktzulassung von qualitativ hochwertigen und gleichzeitig preislich erschwinglichen Generika, da durch den Verzicht auf Studien am Menschen Zeit und Kosten in den Vorbereitungen für eine Zulassung deutlich reduziert werden können. Eine weitere Preisreduktion eines Arzneimittels kann sich

ergeben, wenn mehrere Generika eines Arzneistoffs zugelassen sind und sich dadurch das Marktangebot erhöht. Qualitativ hochwertige generische Arzneimittel sind ein wichtiges Mittel der globalen Gesundheitsversorgung. Eine umfassende Gesundheitsversorgung hängt unter anderem von der Verfügbarkeit von bezahlbaren Arzneimitteln, die den gültigen Qualitätsanforderungen entsprechen, ab. Dies gilt besonders für unentbehrliche Arzneistoffe; umso mehr, wenn diese zur Bekämpfung von Krankheiten mit nur wenigen und/oder teuren therapeutischen Alternativen benötigt werden.

Proguanilhydrochlorid ist ein Arzneistoff, der hauptsächlich in galenisch festen Kombinationspräparaten zur Prophylaxe gegen Malaria eingesetzt wird, während Cefalexinmonohydrat ein antibiotischer Wirkstoff aus der Gruppe der Cephalosporine ist, der oft in festen Formulierungen vorliegt. Beide werden von der WHO zu den unverzichtbaren Arzneistoffen gezählt. Die Biowaiver Monografien beider Wirkstoffe präsentieren neben zahlreichen anderen Informationen die Löslichkeitseigenschaften über einen pH-Bereich von 1-6,8. Da für Proguanil und Cefalexin in der Literatur keine Löslichkeitsdaten für alle regulatorisch geforderten Werten in diesem pH-Bereich vorhanden waren, wurden experimentelle Bestimmungen für beide Substanzen durchgeführt. Zusammen mit der Dosis wurde mit den erhobenen Löslichkeitsdaten für jede Substanz das Dosis/Löslichkeitsverhältnis („dose/solubility ratio“) bei jedem untersuchten pH-Wert berechnet. Gemäß den BCS-Biowaiver Richtlinien der FDA, EMA und WHO muss dieses im pH-Bereich von 1-6,8 unter 250 ml liegen, damit ein Arzneistoff als „hochlöslich“ klassifiziert werden kann. Proguanilhydrochlorid und Cefalexinmonohydrat erfüllen diese Anforderung. Obwohl sich die Stoffe in ihrer Permeabilität unterscheiden (Proguanil: nicht hoch permeabel, daher BCS Klasse III; Cefalexin: hoch permeabel, daher BCS Klasse I), ist damit eine Grundvoraussetzung erfüllt, um generische Produkte beider Wirkstoffe über ein BCS-Biowaiver Verfahren zuzulassen.

Eine Biowaiver Monografie soll eine umfassende wissenschaftliche Betrachtung hinsichtlich der Eignung eines Arzneistoffs und seiner generischen Produkte für das Biowaiver Zulassungsverfahren gemäß den gesundheitsbehördlichen Bestimmungen vorlegen. Dazu gehört die Beurteilung aller verfügbaren Informationen

zu den allgemeinen Eigenschaften des Arzneistoffes (besonders zum Anwendungsgebiet und der Toxizität), zu seinen physikochemischen Eigenschaften, zu seinem pharmakokinetisches Profil, zu bereits zugelassenen Produkten des Arzneistoffs einschließlich Hilfsstoffen, ihrem Freisetzungsverhalten und Berichten über ihre Bio(in)äquivalenz. Wenn Literaturdaten zu Löslichkeit oder dem Freisetzungsverhalten fehlen, unvollständig oder widersprüchlich sind, können Daten zu diesen Eigenschaften durch Experimentalstudien erhoben werden.

Während die Biowaiver Monografien von Proguanilhydrochlorid und Cefalexinmonohydrat ein vollständiges Profil dieser beiden Wirkstoffe und ihrer möglichen generischen Produkte zeigen, stellt die Studie zur Löslichkeit von neuen Arzneistoffen der 16. und 17. Version der EML ein Screening von vielen Stoffen mit verschiedensten Indikationen hinsichtlich ihrer Eignung für ein Biowaiver Verfahren aufgrund ihrer BCS-Klasse dar. Von 16 Arzneistoffen, deren Löslichkeit bei den Schlüssel-pH-Werten 1,2, 4,5 und 6,8 aufgrund von fehlenden Literaturdaten bestimmt wurde, sind neun nach der Definition der regulatorischen Richtlinien „hochlöslich“. Rein im Hinblick auf die BCS-Klasse wären generische, feste Arzneiformen dieser neun Wirkstoffe für ein Biowaiver Zulassungsverfahren geeignet.

Die Arbeit an beiden Projekten verdeutlichte die Relevanz von experimentellen Löslichkeitsversuchen für den Fall, dass eine Recherche in der allgemeinen pharmazeutischen Literatur nicht erfolgreich ist, weil Löslichkeitsdaten für bestimmte pH-Werte nicht verfügbar sind oder verschiedene Quellen sich in den Angaben zur Löslichkeit widersprechen. Das Design solcher Löslichkeitsversuche muss im Kontext von Biowaiver Monografien bestimmte Anforderungen erfüllen. Zum einen müssen durch das Studiendesign die regulatorischen Kriterien zur Bestimmung der Löslichkeitsklasse eines Arzneistoffs erfüllt werden, zum anderen sollte die Methode geeignet sein, um die BCS-Klassifizierung von vielen Arzneistoffen zu realisieren. Dadurch können zukünftige Biowaiver Monografien vorrangig „hochlösliche“ Arzneistoffe berücksichtigen, da aktuell nur diese für ein Biowaiver Zulassungsverfahren geeignet sind.

Die Löslichkeitsmethode, die in dieser Arbeit vorgestellt wird, erfüllt diese Anforderungen. Löslichkeitswerte, die mit dieser Methode erhoben wurden, können bei den Gesundheitsbehörden als Teil einer Zulassungsdokumentation für das BCS-Biowaiver Verfahren eingereicht werden. Gleichzeitig erlaubt dieser Ansatz der Löslichkeitsbestimmung ein Screening vieler Substanzen im Hinblick auf ihre Löslichkeit (und damit ihrer BCS-Klasse) effizient und kostengünstig durchzuführen und verlässliche Daten zu erhalten. Der Ansatz nutzt als Grundlage die im Kleinmaßstab angewendete sogenannte “Shake-Flask”-Methode, mit der die thermodynamische oder Gleichgewichtslöslichkeit bestimmt werden. Die Löslichkeitsexperimente werden mit der höchsten Dosis des Wirkstoffs (höchste Dosisstärke oder höchste therapeutische Einzeldosis) durchgeführt, um zu bestimmen, ob der Arzneistoff nach der Definition der Richtlinien für BCS-Biowaiver „hochlöslich“ ist oder nicht. Wenn die Dosis und ein kleiner Überschuss sich komplett in einem Volumen von 250 ml wässrigem Medium (in diesem Fall: eine proportional kleinere Arzneistoffmenge in einem entsprechend kleinerem Volumen) auflöst, ist der Wirkstoff „hochlöslich“. Die Löslichkeit stellt in diesem Fall eine „Minimallöslichkeit“ dar. Für sehr gut lösliche Substanzen ist das zu erwarten. Detaillierte Erläuterungen des Prinzips der Minimallöslichkeit sind ebenso Teil der vorliegenden Arbeit wie ein Studienprotokoll, von dem eine initiale Version mit den Ergebnissen der Löslichkeitsstudie von neuen Arzneistoffen der 16. und 17. EML veröffentlicht wurde. Die einzelnen Schritte des vollständigen Studienprotokolls werden in der Arbeit ausführlich diskutiert.

Die Methode und das Protokoll beinhalten außerdem den Umgang mit Herausforderungen bei Löslichkeitsstudien wie zum Beispiel Präzipitation oder Degradation. Präzipitationsreaktionen konnten während der Löslichkeitsstudie von Proguanilhydrochlorid beobachtet werden. Mehrere Pufferlösungen, die ausgewählt wurden, um die regulatorisch erforderlichen pH-Bedingungen darzustellen, zeigten sofort bei Zugabe des Wirkstoffpulvers oder später Präzipitate. Folglich war die gelöste Konzentration von Proguanil zum Zeitpunkt der Quantifizierung niedrig. Durch eine andere Auswahl von kompendialen Pufferlösungen konnte die quantifizierte Menge jedoch gesteigert werden, sodass Proguanil final als „hochlöslich“ klassifiziert werden konnte. Weil dieser Fall zeigt, wie entscheidend die Wahl des richtigen Puffermediums für das Ergebnis einer Löslichkeitsstudie

sein kann, bietet diese Arbeit nicht nur eine Aufstellung der gemäß den BCS-Biowaiver Richtlinien geforderten Puffermedien, sondern diskutiert zusätzlich den Einsatz von anderen geeigneten wässrigen Pufferlösungen.

Cefalexinmonohydrat wiederum zeigte während der Löslichkeitsstudie bei bestimmten pH-Werten Zersetzungsreaktionen. Eine nachfolgend durchgeführte Degradationsstudie bei genau diesen pH-Werten ermittelte das Ausmaß in Abhängigkeit von der Zeit und – basierend auf diesen Ergebnissen – einen sinnvollen Zeitrahmen (drei Stunden) für eine ergänzende, verkürzte Löslichkeitsstudie. Die festgelegte Dauer der Zusatzstudie steht im Einklang mit der physiologischen Zeitspanne, die für Einnahme, Auflösung und Absorption einer festen Arzneiform zur peroralen Einnahme in nüchternem Zustand durchschnittlich angenommen wird. Die Ergebnisse dieser zusätzlichen Löslichkeitsstudie zusammen mit denen der Original-Löslichkeitsstudie zeigten, dass Cefalexin entsprechend dem BCS-Biowaiver Richtlinien „hochlöslich“ ist. Weil die Zersetzung im gewählten Zeitrahmen einen Wert von 15 % nicht überstieg, ist die Permeabilitätseinstufung für Cefalexin („hoch permeabel“) nicht beeinträchtigt. Daher wurde Cefalexinmonohydrat final der BCS-Klasse I zugeordnet. Die Herausforderungen, die Zersetzungsreaktionen für eine Löslichkeitsstudie bedeuten, wurden ursprünglich in der Veröffentlichung der Löslichkeitsstudie der EML-Wirkstoffe diskutiert. Entsprechend den Empfehlungen dieser Publikation beinhaltet das Studienprotokoll Details zur Durchführung von Degradations- und zusätzlichen, verkürzten Löslichkeitsstudien und geht darüber hinaus auf Kernaspekte einer stabilitätsindizierenden Analysemethoden ein.

Die vorgestellte Löslichkeitsbestimmungsmethode basiert auf dem Ansatz der „Minimallöslichkeit“ und stellt ein optimiertes Design für Löslichkeitsstudien im Kontext von BCS-Biowaiver Monografien dar. Das dazugehörige Studienprotokoll kann bei Löslichkeitsstudien angewendet werden, deren Ergebnisse den Zulassungsbehörden zusammen mit anderen Daten für eine Zulassung per Biowaiver Verfahren eingereicht werden sollen, aber auch verwendet werden, um die Löslichkeitsklasse vieler Arzneistoffe im Vorfeld von weiteren, detaillierteren Untersuchungen einzelner Wirkstoffe zu bestimmen. Für einen höheren wissenschaftlichen Informationswert einer Löslichkeitsstudie können einzelne Schritte

des Protokolls noch weiter optimiert werden (z.B. Einsatz komplexerer Puffermedien, Feststoffanalytik von Pulverrückständen, Filteradsorptionsstudien), was jedoch die Komplexität der Methode erhöht.

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Figures

Tables

A. Appendix

A.1. Publications

A.1.1. Publication list

Peer-reviewed papers

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. 2018. J Pharm Sci 107(6):1478-1488. (equal first author)

Plöger GF, Abrahamsson B, Cristofoletti R, Groot DW, Langguth P, Mehta MU, Parr A, Polli JE, Shah VP, Tajiri T, Dressman JB. Biowaiver monographs for immediate release solid oral dosage forms: proguanil hydrochloride. 2018. J Pharm Sci 107(7):1761-1772.

Plöger GF, Quizon PM, Abrahamsson B, Cristofoletti R, Groot DW, Parr A, Langguth P, Polli JE, Shah VP, Tajiri T, Mehta MU, Dressman J. Biowaiver monographs for immediate release solid oral dosage forms: cephalexin monohydrate. 2020. J Pharm Sci 109(6):1846-1862.

Posters

Born GF, Nair A, Dressman JB. Qualification of multisource immediate release proguanil hydrochloride products for BCS-based biowaiver. Aug/Sep 2013. FIP World Congress, Dublin, Ireland

Nair A, Born GF, Brinkmann U, Münzenberg J, Dressman JB. Evaluation of feasibility of using AEROPERL® 300 Pharma to enhance solubility of poorly soluble weakly basic APIs. Nov 2013. AAPS Annual Meeting and Exposition, San Antonio, Texas, USA

Born GF, Münzenberg J, Dressman JB. Formulation strategies using AEROPERL® 300 Pharma as a carrier for poorly soluble weakly basic APIs. Apr 2014. FIP Pharmaceutical Sciences World Congress, Melbourne, Australia

Born GF, O´Shea JP, O´Driscoll CM, Griffin BT Dressman JB. Dissolution and in vivo performance of a commercial celecoxib formulation. Oct 2015. AAPS Annual Meeting and Exposition, Orlando, Florida, USA (equal first author)

Plöger GF, Hofsäss MA, Dressman JB. Design of solubility studies in the context of BCS biowaiver monographs. Sep 2018. 12th biennial GPEN conference, Singapore

A.1.2. Publication manuscripts

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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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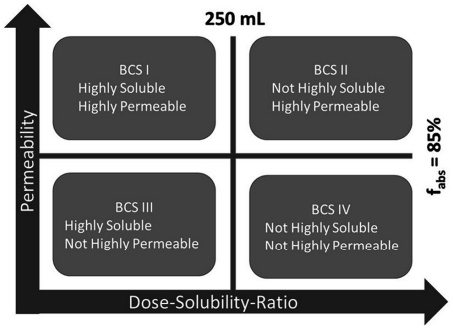

Figure 1. Biopharmaceutics Classification System (modified from Amidon et al.[5]).

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	$66.8 \pm 1.8 \times 10^{4}$	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

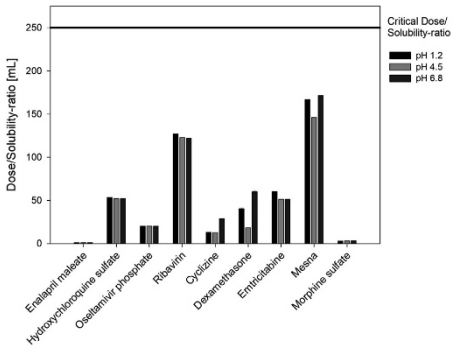

Figure 2. Dose/solubility-ratios of APIs classified as "highly soluble."

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.

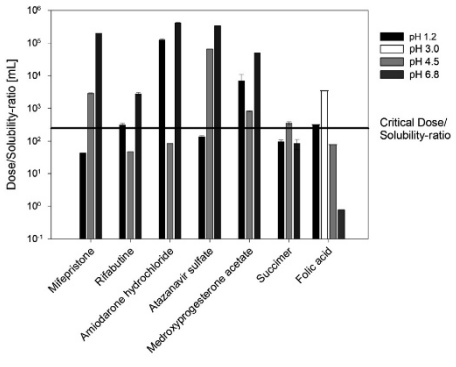

Figure 3. Dose/solubility-ratios of APIs classified as "not highly soluble."

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,[40,40,42,45] 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

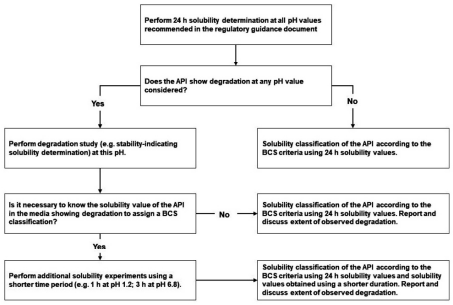

Figure 4. Decision tree for performing solubility determinations in the context of the BCS-based biowaiver.

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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Global Health Commentary

Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Proguanil Hydrochloride

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permeability
regulatory science
solubility

ABSTRACT

Literature data relevant to the decision to waive *in vivo* bioequivalence testing for the approval of generic immediate release solid oral dosage forms of proguanil hydrochloride are reviewed. To elucidate the Biopharmaceutics Classification System (BCS) classification, experimental solubility and dissolution studies were also carried out. The antimalarial proguanil hydrochloride, effective via the parent compound proguanil and the metabolite cycloguanil, is not considered to be a narrow therapeutic index drug. Proguanil hydrochloride salt was shown to be highly soluble according to the U.S. Food and Drug Administration, World Health Organization, and European Medicines Agency guidelines, but data for permeability are inconclusive. Therefore, proguanil hydrochloride is conservatively classified as a BCS class 3 substance. In view of this information and the assessment of risks associated with a false positive decision, a BCS-based biowaiver approval procedure can be recommended for orally administered solid immediate release products containing proguanil hydrochloride, provided well-known excipients are used in usual amounts and provided the *in vitro* dissolution of the test and reference products is very rapid (85% or more are dissolved in 15 min at pH 1.2, 4.5, and 6.8) and is performed according to the current requirements for BCS-based biowaivers.

Introduction

After its discovery in the mid-1940s, proguanil hydrochloride became a widely used prophylactic and therapeutic agent for malaria infections caused by *Plasmodium vivax* and *falciparum*. Only a few years after the first use, resistances to proguanil were detected, especially to *P vivax*. In the 1980s, proguanil aroused new interest as a prophylactic agent in *P falciparum* endemic areas where pyrimethamine resistance appeared.[1] Currently, the World Health Organization (WHO)[2] Guidelines for Treatment of Malaria recommend the use of proguanil hydrochloride in combination with atovaquone (ATO) for causal malaria prophylaxis and for the treatment of uncomplicated malaria in travelers who become infected in areas where malaria is not endemic. In addition, the WHO Model List of Essential Medicines proposes proguanil hydrochloride for malaria prophylaxis in combination with chloroquine.[3]

This biowaiver monograph reviews the literature relevant to decisions to approve new generic immediate release (IR) solid oral products containing proguanil hydrochloride as well as already approved products which have undergone scale-up and post-approval changes ("variations" in European Medicines Agency [EMA] parlance) based on *in vitro* dissolution testing, that is, the Biopharmaceutics Classification System (BCS)–based biowaiver

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procedure, rather than assessing bioequivalence (BE) in clinical studies. This monograph is directed to drug products which contain proguanil hydrochloride as a single active pharmaceutical ingredient (API) and to those which contain proguanil hydrochloride in combination with the antimalarial ATO. Combination products containing proguanil hydrochloride and chloroquine are not available on the market. Clinical, pharmacokinetic, and biopharmaceutical data for proguanil hydrochloride were retrieved from the open scientific pharmaceutical literature. To complement the information found in the open literature, additional solubility and dissolution studies were also performed. Furthermore, the risks associated with applying the BCS-based biowaiver procedure to products containing proguanil hydrochloride are discussed for both individual patients and in the context of public health.

The purpose and scope of the biowaiver monograph series have been explained previously in the literature.[4] Prerequisites for the biowaiver procedure and the approach of conducting a risk/benefit analysis for a given API are laid out in the guidelines from the U.S. Food and Drug Administration (FDA),[5] the WHO,[6] and the EMA.[7] Biowaiver monographs for over 40 APIs with different therapeutic indications have already been published. These monographs are also available on the website of the International Pharmaceutical Federation (http://www.fip.org/bcs_monographs) as well as on the Journal website.

Experimental

Materials

Proguanil hydrochloride pure API (Lot # 50095) was obtained from Molekula (Dorset, UK), whereas the commercial products, Malarone® (Lot # 4671) and Paludrine® (Lot #B90310A) were purchased from Glaxosmithkline (Munich, Germany) and AstraZeneca (Wedel, Germany), respectively. Potassium chloride, potassium dihydrogen phosphate, sodium chloride, and sodium hydroxide were obtained from VWR® Prolabo® (Leuven, Belgium). Acetic acid, hydrochloric acid, and orthophosphoric acid were purchased from VWR® Prolabo® (Fontenay-Sous-Bois, France). All other chemicals were purchased commercially in Germany.

Methods

Literature Research

Literature research was mainly performed in PubMed and Google Scholar. The ROTE LISTE® online,[8] LAUER-TAXE®,[9] and general pharmaceutical literature were also used as sources. Relevant data concerning the therapeutic indication and toxicity of proguanil, its physicochemical and pharmacokinetic properties, all openly accessible BE data, and information about which dosage forms of proguanil are available were collected. Keywords used for the literature search (last updated on February 16, 2017) were proguanil hydrochloride, Malarone, and Paludrine in combination with therapeutic index, solubility, absorption, bioavailability, permeability, distribution, metabolism, elimination, BE, excipients, interaction, and ATO.

Solubility Studies

The solubility of proguanil hydrochloride was evaluated using a modified shake-flask method over a pH range of 1-6.8 ($n = 3$ for each pH studied). The following buffers were prepared for this purpose: potassium chloride/hydrochloric acid solutions according to the United States Pharmacopeia (USP[10]) at pH 1.0 and 1.2, USP potassium chloride/hydrochloric acid buffers at pH 1.3 and pH 2.3 (corresponding to 1 pH unit below the pK_a and the pK_a of proguanil, respectively),[10] an adjusted phosphate buffer at pH 3.3 of the European Pharmacopoeia (Ph. Eur.)[11] (corresponding to 1 pH unit above the pK_a), an acetate buffer solution at pH 4.5 from the Ph. Eur.,[11] and a USP phosphate buffer at pH 6.8.[10] Since preliminary experiments indicated that proguanil hydrochloride has a very high solubility, the aim of the experiments was to determine whether the highest dosage strength and the highest single therapeutic dose are both soluble in a volume of 250 mL or less at the various pH values, rather than determining the thermodynamic solubility. An amount of ~12 mg was weighed accurately into each of 3 Uniprep™ vials (Whatman™, Little Chalfont, UK). Three milliliters of pH 6.8 phosphate buffer was added, resulting in a maximum concentration of ~4 mg/mL, equivalent to 1000 mg per 250 mL. Thus, if the entire ~12 mg dissolved, this demonstrated that the BCS criterion (D:S $\leq$ 250 mL) had been met. The procedure was also carried out at pH 4.5, 3.3, 2.3, 1.3, 1.2, and 1.0. Vials were gently shaken in an orbital shaker (POLYMAX 1040; Heidolph Instruments, Schwabach, Germany) at 37 ± 0.5°C for 24 h. Afterward, the samples were filtered through the Uniprep™ filter (PTFE membrane, pore size 0.45 μm), the pH of the buffer was recorded, and the filtrate was diluted appropriately for analysis.

The concentration of dissolved drug in each sample was first analyzed by ultraviolet (UV) spectroscopy. Because of the high variability in the results, the solubility experiments were repeated, and the concentration of all samples was analyzed by high-performance liquid chromatography with UV detection. The system consisted of a Hitachi LaChrom Elite® pump (L-2130), an autosampler (L-2200), and an UV-detector (L-2400) as well as a column oven (VDS optilab). A LiChrospher® 100 RP-18 (5 μm) LiChroCART® 125 × 4 cartridge (Merck Milipore, Darmstadt, Germany) was used for analysis. The mobile phase was composed of methanol and a phosphate buffer pH 5.8 (1:1). The flow rate was set at 1 mL/min, the column temperature at 60°C, and the run time at 10 min. The injection volume was 20 μL. The retention time of proguanil hydrochloride in this set up was approximately 4 min. The detection wavelength was 232 nm. The high-performance liquid chromatography method was validated in accordance with the International Conference on Harmonisation guideline Q2(R1)[12] with respect to linearity, repeatability, detection limit (DL, ~0.79 μg/mL), and quantitation limit (QL, ~2.4 μg/mL).

In Vitro *Dissolution Studies*

The dissolution studies were performed using an Erweka® Dissolution Tester (DT80, USP Apparatus II; Erweka GmbH, Heusenstamm, Germany). The dissolution behavior of the pure API and 2 commercial products, Malarone® and Paludrine® was evaluated in 900 mL medium at an agitation speed of 75 rpm at 37 ± 0.5°C, in sextuplicate. Simulated gastric fluid without pepsin (SGF_{sp}, USP)[10] at pH 1.2, an acetate buffer at pH 4.5 (USP),[10] and dissolution buffer pH 6.8 TS (International Pharmacopoeia [Int. Ph.])[13] were used as dissolution media. The WHO requires that buffers from the Int. Ph. or other suitable buffers with the same buffer capacity be used for dissolution tests.[6] The guideline also requires the use of pH 1.2 hydrochloric acid buffer or solution and pH 4.5 acetate buffer. Because the chapter "Dissolution tests for solid oral dosage forms" in the Int. Ph. does not contain a solution with pH 1.2 or an acetate buffer pH 4.5,[13] standard USP buffers with a similar buffer capacity were chosen for these 2 pH values. The Biowaiver guidances require different values for the agitation speed for the paddle apparatus (FDA: 50 or 75 rpm,[5] WHO: 75 rpm,[6] and EMA: usually 50 rpm[7]). Because a paddle speed of 75 rpm was shown to reduce coning of the commercial products (unpublished data from preliminary testing), it was deemed to be the most suitable paddle speed. The temperature and the pH of the medium were checked before and after the dissolution test. A 1 mL sample was withdrawn at 5, 10, 15, 20, 30, 45, and 60 min and replaced with 1 mL of fresh medium,

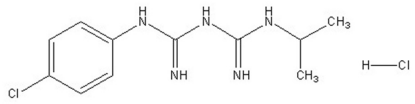

Figure 1. Structure of proguanil hydrochloride.

prewarmed to 37 ± 0.5°C. The filtered samples (Whatman® PTFE syringe filter 0.45 μm; Whatman GmbH, Dassel, Germany) were diluted for further analysis, and their absorbance was measured against suitable blanks by UV spectrophotometry (Hitachi U3000 Spectrophotometer) at 250 nm wavelength. This method of analysis was chosen in accordance with the USP, which recommends UV spectroscopy for dissolution testing of other antimalarials, for example, pyrimethamine tablets[14] despite a potential interference by soluble excipients.

General Characteristics

Nomenclature and Structure

The International Nonproprietary Name of proguanil is proguanil.[15] The chemical names for the base are N^1-4-chlorophenyl-N^5-isopropyldiguanide,[15] N-(4-Chlorophenyl)-n'-(isopropyl)-imidodicarbonimidic diamide,[16] and N-(4-Chlorophenyl)-N'-(1-methylehtyl)imidodicarbonimidic diamide.[17] The chemical names for the hydrochloride salt are 1-(4-Chlorophenyl)-5-(1-methylethyl)biguanide-hydrochloride[18] and 1-(4-Chlorophenyl)-5-isopropylbiguanide hydrochloride.[19] Various synonyms are known for proguanil, for example, chloroguanide hydrochloride or proguanide hydrochloride.[19] The CAS number for proguanil base is 500-92-5 and for proguanil hydrochloride 637-32-1.[18,19]

Proguanil hydrochloride has the molecular formula $C_{11}H_{17}Cl_2N_5$ with a molecular weight of 290.2 g/mol. Its structure is shown in Figure 1.

Therapeutic Indication and Dosing

Because of the prevalence of resistance, proguanil hydrochloride is not used for either the prophylaxis or treatment of malaria as single therapy.

Prophylaxis

Proguanil hydrochloride is recommended for prophylaxis of malaria in combination with ATO. The combination is mainly used as causal prophylaxis (inhibition of the liver-stage development of the parasite) since proguanil and ATO are both active against the preerythrocytic stages of the parasite.[2] Unmetabolized proguanil enhances the activity of ATO.[20] Proguanil is also active against the erythrocytic stage via its metabolite cycloguanil. As ATO also demonstrates a high activity against the asexual stage of *Plasmodium in vitro*,[21] the combination of both substances can be additionally considered for suppressive prophylaxis (inhibition of blood stages). Four tablets of the fixed-dose combination (FDC) with ATO, for example, Malarone® equal to a total of 400 mg proguanil hydrochloride are given once daily for prophylaxis. The intake is recommended to start 24 to 48 h before an exposure, should be maintained during duration of exposure, and can be terminated a week after leaving the endemic area.[22,23]

The WHO Model List of Essential Medicines lists proguanil hydrochloride as a prophylactic agent in combination with chloroquine,[3] although FDCs of these 2 APIs are not commercially available. An evidence-based prophylaxis or therapy guideline for this combination could not be found in the open literature. According to the prescribing information for the single API preparation, Paludrine®,[23] 2 tablets are administered daily. This is equal to a daily dose of 200 mg proguanil hydrochloride. Prophylaxis treatment should be started 1 week before entering an endemic area, should be maintained during exposure, and can be stopped 4 weeks after leaving the area.

Treatment

The combination of proguanil hydrochloride and ATO is also used for the treatment of uncomplicated malaria caused by *P falciparum* in those traveling in nonendemic areas.[2,22] *Plasmodium knowlesi*, a South-Eastern Asian pathogen variety, showed sensitivity to the combination of proguanil hydrochloride and ATO, whereas *P vivax* is resistant against both the combination and proguanil hydrochloride alone.[2,23] Proguanil hydrochloride and ATO can be combined with artesunate and primaquine for the therapy of uncomplicated malaria, if other combinations are not available or in case of resistance. A treatment of uncomplicated *falciparum* malaria requires a daily intake of 4 tablets of the FDC with ATO (equal to 400 mg proguanil hydrochloride) on 3 consecutive days.

Special Populations

Children and pregnant women are 2 population groups who are particularly vulnerable to malaria infections.[24-26] Especially pregnant women need an effective and safe malaria prophylaxis. The combinations proguanil/ATO and proguanil/chloroquine are both regarded as safe during pregnancy, the latter being especially suitable in the first trimester.[2,25]

Children, as well as others with a body weight below 40 kg, need adjusted dosage schedules. The combination of proguanil hydrochloride and ATO is also available in low-dose tablets equal to 62.5 mg ATO and 25 mg proguanil hydrochloride. These tablets are taken according to adjusted dosing schemes especially for prophylaxis.[27,28] An adjusted dosing regimen for children is also available for single API preparations of proguanil hydrochloride.[23]

Therapeutic Index and Toxicity

Toxicity and Adverse Effects in Animals

Single-dose toxicity studies were conducted by Butler et al., determining median lethal dose (LD_{50}) values of 60-80 mg/kg and 100-150 mg/kg in mice and rats after oral administration, respectively. Values for the maximum tolerable dose (LD_0) were found to be 50 and 80 mg/kg after oral administration, respectively.[29] Repeated oral administration of 2.9 mg/kg body weight for 5 days and 6 weeks, respectively, resulted in reversible effects on reproduction function of male rats.[30]

Adverse effects after chronic (6 months) administration of proguanil hydrochloride to laboratory animals are mainly observed at multiples of the doses used in human prophylactic and treatment regimens, which are 2.9 and 5.7 mg/kg per day, respectively.[31] These include mucosal hyperplasia of the cecum and renal tubular basophilia (rats treated with 20 mg/kg per day), bile duct hyperplasia, gall bladder mucosal atrophy, interstitial pneumonia, fibrovascular proliferation in the right atrium, pyelonephritis, bone marrow hypocellularity, lymphoid atrophy, and gastritis/enteritis (dogs treated with 4-12 mg/kg per day).

Table 1
Minimum Solubility of Proguanil Hydrochloride in Different Buffer Solutions With pH Values From 1.0 to 6.8 at 37 ± 1°C

Medium	Initial pH	Final pH	(Minimum) Solubility (mg/mL)	(Maximum) D/S Ratio (mL), D = 200 mg	(Maximum) D/S Ratio (mL), D = 400 mg
Hydrochloric acid buffer[a]	1.0	1.1	4.3 ± 0.4	47	93
Hydrochloric acid buffer[b]	1.2	1.3	4.21 ± 0.11	48	95
Hydrochloric acid buffer[b]	1.3	1.4	4.1 ± 0.9	49	97
Hydrochloric acid buffer[b]	2.3	2.8	5.3 ± 0.4	38	75
Phosphate buffer[c]	3.3	3.4	2.58 ± 0.26	78	155
Acetate buffer[d]	4.5	4.6	4.7 ± 0.6	42	85
Phosphate buffer[b]	6.8	6.8	2.89 ± 0.06	69	138

[a] Standard buffer solution (USP) with pH adjusted to initial pH.
[b] Standard buffer solution (USP).
[c] Buffer solution (Ph. Eur.) with pH adjusted to initial pH.
[d] Buffer solution (Ph. Eur.).

Toxicity and Adverse Effects in Humans

Symptoms of overdosage include hematuria, renal irritation, epigastric discomfort, and vomiting.[23,31] Overdoses of proguanil hydrochloride as large as 1500 mg have been followed by complete recovery, and doses as high as 700 mg twice daily have been taken for over 2 weeks without serious toxicity.[31]

Undesirable effects of proguanil hydrochloride in humans are gastrointestinal disorders such as diarrhoea and constipation, mouth ulceration and stomatitis, cholestasis, skin reactions as skin exfoliation (scaling of the skin on the palms and soles), rash, pruritus and reversible alopecia, fever, hypersensitivity, including urticaria, angioedema, vasculitis, and acute eosinophilic pneumonia, hematological changes such as aplastic anemia, anemia megaloblastic, and pancytopenia (frequency unknown).[23,31,32] Pancytopenia as a severe adverse effect has been reported in conjunction with impaired renal function exacerbated by dehydration.[33] Time- and dose-dependent decreases in viability of lymphocytes, observed at 2 different plasma concentrations *in vitro*, were reported by Gajski et al.[34] The use of proguanil hydrochloride, similar to several other antimalarials, also increases the risk of developing ophthalmic disorders. Slightly increased incidence rates for different ophthalmic disorders for proguanil as part of a combination with chloroquine or ATO have been reported.[35]

Proguanil hydrochloride is not mentioned in the FDA Guidance for Scale-Up and Post-Approval Changes from 1995, which lists generally known narrow therapeutic index drugs in Appendix A of the study conducted by FDA.[36] Because severe adverse effects are rare and overdoses are tolerated even over a longer period without serious toxicity, proguanil hydrochloride can be regarded as a drug substance with a broad therapeutic index. Nevertheless, it is strongly recommended to use this antimalarial drug according to the prescribing information, which requires a glomerular filtration rate greater than 30 mL/min. In addition, sufficient hydration is strongly recommended during the intake of proguanil hydrochloride.

Physicochemical Properties

Salts, Polymorphs, Solvates, and Hydrates

Initially, proguanil was used in the acetate form[37] but was later replaced by the more easily synthesized hydrochloride salt.[38] Proguanil is currently listed as the hydrochloride salt in the European, the International and the USP.[18,39,40] The European Pharmacopoeia first mentioned it in the fourth edition (fourth supplement, 2003),[41] while the USP published a proguanil hydrochloride monograph for the first time in the USP 33 Reissue (2010).[42] Proguanil hydrochloride is commercially available in pharmaceutical formulations as the hydrochloride salt.[43] Polymorphs, solvates, and hydrates have not been discussed to date in the open literature.

Solubility

The European Pharmacopoeia[44] indicates that proguanil hydrochloride is slightly soluble in water. Other pharmaceutical literature reports the water solubility to be around 1 percent,[17,43] which is concordant with the assessment of the Pharmacopoeia. The FDA Biowaiver Guidance[5] recommends standard buffer solutions from the USP as media for solubility testing. The USP describes acid phthalate buffers as standard buffer solutions for pH values 2.3 and 3.3. However, pilot studies revealed a tendency of proguanil hydrochloride to flocculate in the presence of counterion buffer components, including phthalate and maleate. For this reason, pH 2.3 and 3.3 phosphate buffer solutions from the European Pharmacopoeia were chosen for the solubility studies. Precipitates of crystallized proguanil phosphate were also detected in these media, although only to a minor degree. Table 1 shows the results of solubility studies at different pH values, together with related dose/solubility (D/S) ratios for highest strength and highest single therapeutic dose of proguanil hydrochloride, respectively.

Partition and Distribution Coefficient

Clarke's[17] reported a log P in octanol/water of 2.5 for proguanil without specifying whether this value was experimental or calculated. The Drug Bank lists an experimental log P of 1.89 and a predicted log P of 1.9 using modeling software.[16] In addition, a Clog *p* value of 1.442 was found in the literature.[16] A log D of -4 at pH below 7 has been reported with reference to modeling software.[45] Experimental values for log D could not be found in the open literature.

pK_a

The commentary to the European Pharmacopoeia,[41] as well as Clarke's Analysis of Drugs and Poisons,[17] records a value of 2.3 as pK_{a1} and 10.4 as pK_{a2} at 22.5°C for both imine groups of the basic biguanide function of proguanil. Another source in the open pharmaceutical literature reports the same pK_a values at 25°C.[46] Lindegardh et al.[45] reported values for pK_a of 12.6 (pK_{a1}) and 10.6 (pK_{a2}), but these values were predicted with Pallas 3.0 software rather than being experimentally determined.

Dosage Form Strengths

Dosages of proguanil always refer to the hydrochloride salt. In some countries, proguanil hydrochloride is available as a single API

IR solid oral dosage form, for example, Paludrine® 100 mg tablets, corresponding to 87.44 mg of the proguanil base. Since proguanil hydrochloride is not administered alone for resistance reasons, FDCs of proguanil with ATO are also available[2,3] for example, Malarone® consisting of 250 mg ATO and 100 mg proguanil hydrochloride (equal to 87.44 mg proguanil base). Malarone® is also available as a pediatric dosage form with 62.5 mg ATO and 25 mg proguanil hydrochloride. Generic products of both dosage strengths are available on the market. This monograph is directed to both the proguanil component of FDC and to products containing proguanil hydrochloride as the single API.

Pharmacokinetic Properties

Proguanil is metabolized to the active metabolite cycloguanil[47] and an inactive metabolite p-chlorophenylbiguanide (CPB) via liver enzymes,[41,48] but it cannot be considered as a simple prodrug because proguanil itself is also active against malaria strains. *In vitro*, proguanil is less active than cycloguanil.[48] The metabolite inhibits the dihydrofolate reductase of the parasite,[21] to a much greater extent than proguanil.[48] *In vivo*, proguanil shows a higher antimalarial activity than the metabolite cycloguanil. This activity is partly because of the continuous metabolism of proguanil to cycloguanil,[48] which is barely absorbed.[49] Another part of the activity of proguanil is based on its antimalarial effects in combination with another antimalarial drug substance.[20,21] Specifically, proguanil enhances the ability of ATO to collapse the mitochondrial membrane potential in malaria parasites, whereas cycloguanil has no enhancing effect on the activity of ATO.[20]

Bioavailability and Absorption

Values for absolute bioavailability have not been reported in the open literature to date. Although Maegraith et al.[38] described experiments with both intravenous and oral application of proguanil hydrochloride, specific pharmacokinetic data, that is, values for the area under the curve (AUC) or the maximum concentration (C_{max}), were not reported. Other authors published pharmacokinetic results based on studies only after oral administration (see in the following).

The first pharmacokinetic results for proguanil hydrochloride were published in the 1940s by Maegraith et al.[38] Proguanil is rapidly absorbed, showing peak plasma concentrations within 2-4 h after single dose or repeated administration.[1,50-52] An apparent first order absorption rate constant of 0.64 ± 0.17 h^{-1} after administration of single doses of 200 mg to healthy adults can be found in the literature, leading to a mean apparent absorption half-life for proguanil of 68 ± 16 min.[1] Similarly, Hussein et al.[53] reported a final population estimate for k_a in patients with acute *P falciparum* infection of 0.513 h^{-1} after combination therapy with ATO, leading to a population absorption half-life of 1.35 h. After a single dose of 200 mg proguanil hydrochloride, Wattanagoon et al.[1] determined a C_{max} of 150-220 ng/mL for proguanil and 12-69 ng/mL for cycloguanil. AUC values for proguanil and cycloguanil have been reported as 3046 ± 313 and 679 ± 372 ng/mL·h (mean values plus/minus standard deviation), respectively. Similar values were found in the study by Meyer et al.[54] under steady-state conditions.

C_{max} and AUC values vary with respect to the metabolic status of the patient. Thapar et al.[52] reported C_{max} values for proguanil and its main metabolite cycloguanil of 218-393 nM and 42-340 nM, respectively, after single doses of 100 mg proguanil hydrochloride in extensive metabolizers (EM). Steady-state levels of C_{max} of 261-735 nM for proguanil and 65-335 nM for cycloguanil were also reported in this study. Median AUC values in h·μM of 3.54 (proguanil) and 3.04 (cycloguanil) and 5.26 (proguanil) and 3.17 (cycloguanil) have been reported for single dose and repeated dose administration, respectively.

After single doses of 50 to 500 mg proguanil hydrochloride, 40%-60% was recovered in urine as parent and metabolites, whereas 10% was excreted in the feces as parent and metabolite.[38] In a review article, Smith et al.[55] reported similar results, with a urinary recovery of 59% after oral administration of 300 mg of proguanil, consisting of 62% proguanil, 30% cycloguanil, and 8% CPB. Edstein et al.[56] reported a somewhat lower recovery of 35.6% ± 9.6% of an orally administered dose of 200 mg proguanil as parent and the primary metabolite, cycloguanil, in the urine at steady-state conditions.

Maegraith et al.[38] demonstrated dose-proportionality for the absorption process with doses of 50 to 500 mg administered orally to healthy adults. Similarly, Hussein et al.[53] concluded that the pharmacokinetics in malaria patients is linear for doses from 100 to 400 mg because an increase in dose did not affect oral (= total) clearance, metabolism, or protein binding.

The absorption of proguanil is not dependent on food intake.[31] Similarly, coadministration of chloroquine or ATO had no impact on the absorption of proguanil.[52,54] For diarrhoea patients, a significantly lower C_{max} and a longer T_{max} were reported, whereas the absorption coefficient (in mL/h) was not significantly lower.[57]

Pharmacokinetic characteristics are changed in pregnancy due to increased oral clearance and volume of distribution (V_d). After single doses of 200 mg to healthy pregnant women in the third trimester, values for C_{max} and AUC of cycloguanil were markedly decreased.[26] Pregnant women with acute *falciparum* malaria treated with a combination of ATO, proguanil hydrochloride, and artesunate for 3 days showed lower plasma concentrations for proguanil.[58] In another study, third-trimester pregnant women with acute uncomplicated malaria (quinine treatment failure) treated with Malarone® for 3 days showed an approximately 2-fold decrease in both C_{max} and AUC of proguanil.[25]

Permeability

Bergström et al.[59] evaluated the permeability of proguanil in Caco-2 cell culture experiments grown on Transwell filters. P_{app} was published in their conference poster to be 0.69 ± 0.02 × 10^{-6} cm/s in the basolateral (A→B) direction and 4.46 ± 1.26 × 10^{-6} cm/s in the apical (B→A) direction (without details on the reference compound). Although B→A/A→B ratio in Caco-2 cells is not directly translatable to the upper intestine in humans, the results suggest that proguanil may to be subject to an efflux mechanism. According to a correlation of P_{app} (determined in Caco-2 cells on polycarbonate filters) and the extent of oral drug absorption in humans obtained by Artursson et al.,[60] an apparent permeability coefficient between 0.1 and 1.0 × 10^{-6} cm/s indicates that a drug substance is incompletely absorbed in humans. According to the Artursson correlation, completely absorbed drugs would show an absorption coefficient greater than 1.0 × 10^{-6} cm/s, whereas drugs with an extent of absorption less than 1% would show a lower permeability coefficient. On this basis, Bergström et al.[59] concluded that the permeability of proguanil corresponds to less than 85% absorption in humans.

A literature search on *in vivo* pharmacokinetic or intestinal permeability studies of proguanil revealed no results. For cycloguanil, no permeability data of any kind could be located in the open literature.

Distribution

Several publications indicate a higher concentration of proguanil in whole blood compared to plasma, with an attendant

accumulation of the drug in erythrocytes.[1,51] Wattanagoon et al. measured a 5-fold higher concentration of proguanil in whole blood than in plasma after an oral single dose of 200 mg to healthy subjects. The inactive metabolite CPB was 4 times higher in whole blood than in plasma whereas levels of cycloguanil in plasma and whole blood were similar. Similar results were found by Bygbjerg et al.[51] *in vitro* and *in vivo*.

Maegraith et al.[38] published a plasma protein binding value for proguanil of 70%-80%.

Values for the volume of distribution (V_d) and the volume of distribution related to the bioavailability (V_d/F), respectively, vary according to the literature source. Watkins et al. determined the volume of distribution in healthy subjects after a single dose administration of 200 mg for poor and extensive metabolizers,[61] reporting a value of 856 ± 468 L for extensive metabolizers and 1420 ± 873 L for poor metabolizers based on plasma concentrations. Kelly et al.[50] reported a volume of distribution of 21.03-25.64 L/kg during prophylaxis, without indicating whether concentrations in whole blood or plasma were used as the reference point. A population pharmacokinetic study in malaria patients from different ethnic groups treated with proguanil and ATO revealed a relationship between apparent volume of distribution (determined in plasma) and body weight, yielding a population estimate of 1629 L for patients aged greater than 15 years with a mean body weight of 54.8 kg.[53]

An increased volume of distribution in pregnancy was observed by McGready et al. in 2003 and Na-Bangchang et al. in 2005. The pharmacokinetics in pregnant women after a 3-day treatment with ATO and proguanil hydrochloride was determined in both publications. V_d/F was reported as 22.9 ± 1.4 L/kg and 10.7-34.0 L/kg, respectively.[25,58]

Metabolism

Proguanil is metabolized to cycloguanil and p-chlorophenylbiguanide in the liver. Both metabolites were indentified early after the discovery of the antimalarial.[47] For a long period, cycloguanil was regarded as the API since *in vitro* studies and *in vivo* observations with different types of laboratory animals demonstrated a higher activity for the metabolite, noting that the first investigations focused on strains of *Plasmodium gallinaceum* and *cynomolgi*.[55] *In vitro* studies conducted by Watkins et al.[48] confirmed the high activity of cycloguanil with lower mean inhibitory concentrations (IC_{50}) against different strains of *P falciparum* compared with proguanil and the second metabolite CPB. But subsequent research revealed synergistic effects only for the parent compound proguanil in combination with the antimalarial ATO.[20-22]

Proguanil is primarily metabolized via liver enzymes of the cytochrome P450 family, especially by S-mephenytoin hydroxylase (which is also known as CYP2C19).[62] Metabolism by CYP3A enzymes has also been reported in the literature.[63] Differences in the metabolism of proguanil to cycloguanil were identified by Ward et al.[64] in 1989 after earlier reports on intersubject variabilities of cycloguanil concentrations in plasma. They showed a correlation between the proguanil/cycloguanil ratio in urine and plasma after a single dose of 200 mg of proguanil at the 6-h sampling point in healthy adults at steady state. A cutoff ratio of 10 was used as a criterion to differentiate between extensive metabolizers (ratio <10) and poor metabolizers (ratio >10). Recent studies identified the alleles 2 and 3 as common defective variants, and variations in the 5' regulatory region as the reason for a low metabolic ratio.[65]

The percentage of poor metabolizers varies in different ethnic groups.[53] Several studies evaluating the ratio of extensive and poor metabolizers in population groups can be found in the literature.[61,64,66] For example, Ward et al.[64] studied the metabolic variability in a population of 135 British troops during prophylaxis, reporting 10% of the group to be poor metabolizers, whereas Watkins et al.[61] determined 35% of a group of 65 healthy Kenyan adults to be poor metabolizers. Reduced biotransformation was also observed during pregnancy and in women using oral contraceptives.[50,67]

Maximum plasma concentrations of cycloguanil appear approximately 5 h after a single dose administration of the parent drug.[1,52] At steady-state conditions with a dose of 200 mg proguanil, Bygbjerg et al. reported a cycloguanil plateau after 3 h, whereas Thapar et al. observed maximum concentrations after about 5 h for doses of 100 mg.[51,52] Similarly, after repeated administration of 200 mg proguanil hydrochloride in different regimens, cycloguanil showed maximum concentrations at 4.5-4.9 h.[54] Peak plasma concentrations of the second metabolite, CPB, occur approximately 6 h after administration.[1] All 3 substances showed parallel plasma concentration profiles, with a phase shift for the 2 metabolites.

Changes in pharmacokinetic characteristics, including the parameters C_{max} and AUC, were measured in poor metabolizers by Thapar et al.[52] Under steady-state conditions, values for AUC of proguanil were slightly increased in poor metabolizers from 3.29-9.10 h·μM (extensive metabolizers) to 3.88-11 h·μM (poor metabolizers), whereas the AUC of cycloguanil was decreased in poor metabolizers from 0.52-4.69 to 0.03-0.38 h·μM. Similarly, values for C_{max} of proguanil were determined to be 261-735 nM in extensive metabolizers and 533-832 nM in poor metabolizers, whereas Cmax values for cycloguanil were 65-355 nM (extensive metabolizers) and 6.15-79.8 nM (poor metabolizers). These results indicate that the antimalarial benefit in poor metabolizers is mainly based on proguanil rather than cycloguanil.

Elimination

Renal elimination is the main excretion pathway for proguanil with a renal clearance of about 25% of the oral clearance (which is the apparent total clearance of the drug from plasma after oral administration).[1,56] Single-dose pharmacokinetic studies showed a renal clearance for proguanil of 5.0 ± 1.9 mL/min·kg and for cycloguanil of 4.1 ± 1.8 mL/min·kg and an oral clearance for proguanil of 19.1 ± 2.8 mL/min·kg.[1] Studies with the same dose at steady-state revealed a renal clearance of 0.33 ± 0.19 L/h·kg[56] (proguanil) and 0.35 ± 0.15 L/h·kg (cycloguanil) and an oral clearance for proguanil of 1.43 ± 0.33 L/h/kg. These values are similar to the single-dose pharmacokinetic data. Neither study indicated whether the data were obtained from extensive or poor metabolizers.

An increase in oral clearance has been reported for pregnant women with acute *falciparum* malaria,[58] which is attributed to a higher renal clearance since the biotransformation seems to be reduced in pregnant women.

Proguanil has a mean residence time in the body of approximately 20 h.[1] The mean terminal elimination half-life of around 16 h reported by Wattanagoon et al. is comparable to reports by other authors.[1,25,38,51,53,54] The elimination profiles of proguanil and cycloguanil are similar and described as parallel.[1,25,61] Initial assumptions that cycloguanil is cleared more rapidly by the kidneys[55] were refuted by Watkins et al. in 1990. The elimination half-life of proguanil in plasma of 11.5 ± 2.9 h after application of 200-mg proguanil to extensive metabolizers is shorter than the cycloguanil half-life of 21.1 ± 12 h.[61] Thapar et al. characterized the elimination half-life of proguanil and cycloguanil among other pharmacokinetic criteria in extensive and poor metabolizers after single doses and at steady-state with ATO/proguanil. In extensive metabolizers,

proguanil showed a longer half-life at steady-state (13.1 h) than after a single dose (8.03 h). The same trend was observed for poor metabolizers, with a half-life of 14.5 h at steady-state compared to 9.94 h after a single dose. For cycloguanil, an elimination half-life of 8.09 h was reported in extensive metabolizers at steady-state compared with 10.2 h after a single dose. In poor metabolizers, the cycloguanil elimination half-life was determined to be 11.1 h at steady-state and 8.96 h after a single dose.[52] The decreased elimination half-life of cycloguanil in extensive metabolizers at steady-state could result from an increased clearance of the metabolite.[52] Explanations for these results, for example, an induction of the clearance of the metabolite, have not been confirmed.

Dosage Form Performance

BE Studies

Literature research on BE issues retrieved no results. However, several generic products corresponding to the FDC product Malarone® are available commercially in International Conference on Harmonisation and associated countries, indicating that bioequivalent products are being manufactured.

Excipients

Table 2 specifies the excipients used in representative proguanil hydrochloride or proguanil/ATO combination IR products with a marketing authorization (MA) in Austria, Belgium, Canada, Switzerland, Czech Republic, Germany, Denmark, Spain, Finland, France, Greece, Hungary, Ireland, Italy, The Netherlands, Norway, Poland, Portugal, Sweden, Slovakia, the United Kingdom, and the United States. No proguanil hydrochloride products are available on the WHO Prequalification List.[68]

Many products are on the market, most of which are generic products of the proguanil/ATO combination product Malarone®. None of the excipients presented in the table is known to affect the gastrointestinal motility or transit time, either of which could lead to reduced absorption. Excipients that could interact with membrane transporters, for example, inhibit efflux transporters and lead to an increased permeability are also absent from the listed products. No reports of excipient interactions with proguanil hydrochloride or of a different absorption/bioavailability or permeability for the drug substance caused by excipients could be found in the open literature.

Dissolution

Dissolution studies were performed with the pure API, a marketed product containing 100 mg proguanil hydrochloride (Paludrine®, equal to 87.44 mg base) and a marketed combination product containing a fixed dose of 250 mg ATO and 100 mg proguanil hydrochloride (Malarone®, equal to 87.44 mg base) at pH values of 1.2, 4.5, and 6.8, in triplicate. Figure 2 shows the results of the dissolution studies. These demonstrated that for both pure drug substance and drug products more than 85% of the labeled amount of the API was dissolved within 15 min at all 3 pHs.

Discussion

Solubility

Literature solubility data for proguanil hydrochloride were based on determinations in water at room temperature. To provide data on the pH-dependent solubility behavior of proguanil, BCS confirmatory solubility studies were performed at 37°C in accordance with the guidelines of FDA, WHO, and EMA. Because a pK_a value of 10.4 indicates that proguanil has a relatively strong basic function at one of the imine groups, proguanil hydrochloride will be protonated over the entire BCS relevant pH-range of 1.0-6.8. The other imine group, with a pK_a of 2.3, is fully protonated only under strongly acidic conditions. Because one of the 2 pK_a values lies between 1.0 and 6.8, FDA[5] and EMA[7] require the solubility to be determined at that p*Ka*. As the more basic p*Ka* ensures ionization over the entire BCS relevant range, a pH dependency of solubility in this range was neither expected nor observed.

The FDA guidance requires D/S ratios based on the highest strength, whereas the WHO and EMA guidelines demand a calculation based on the highest single therapeutic dose listed by the regulatory authority and the highest single dose recommended in the Prescribers' Information, respectively. Proguanil hydrochloride demonstrated D/S ratios less than 250 mL over the pH range of 1.0-6.8 (Table 1), for both the highest dosage strength (100 mg) and the highest single dose, which is 400 mg. These results indicate that proguanil is "highly soluble" according to all 3 BCS guidances.

Permeability

Although "extensive absorption" is described by several publications, fraction absorbed and permeability data to assess whether the fraction absorbed is more or less than 0.85 are insufficient to determine whether proguanil is a highly permeable substance or not.

There is also the question of whether proguanil or cycloguanil permeability should be measured, since cycloguanil is an active metabolite of proguanil. In fact, the *in vitro* mean inhibitory concentration of proguanil is several thousand times higher than that of cycloguanil,[21,48] whereas blood levels are about 2 times higher in extensive metabolizers and more than 20 times higher in poor metabolizers (calculation based on C_{max} values at steady-state conditions with 100 mg proguanil hydrochloride daily[52]). According to the FDA Biowaiver Guidance, the permeability of the parent compound (prodrug) should be measured in such cases.[5] However, in the case of proguanil, the parent compound shows significant antimalarial activity. Unlike cycloguanil, proguanil specifically enhances the activity of ATO in malaria parasites.[20,21] In addition, the oral administration of proguanil, with its continuous metabolism to cycloguanil, ensures higher plasma levels of cycloguanil than after an oral administration of cycloguanil, which according to a review of N. White[49] in 1985 is only poorly absorbed (supporting data not reported). For these reasons, proguanil is more active against malaria *in vivo* compared to cycloguanil and cannot be considered to be merely a prodrug of cycloguanil.

In vivo absolute bioavailability or intestinal permeability data for proguanil could not be found in the open literature, although several pharmacokinetic studies have shown that a dose of proguanil is excreted to an extent of approximately 60% in urine. A permeability determination in Caco-2 cells predicted an incomplete absorption of proguanil of about 50% based on a previous correlation of apparent permeability coefficient and absorption in humans.[59] This finding is consistent with the high, basic p*Ka* value of proguanil (10.4). As proguanil is fully protonated at intestinal pH, and therefore exhibits a lower log D value than for the corresponding unionized form, its ability to penetrate the intestinal membrane is expected to be suboptimal. The authors also discussed the possibility of proguanil being a substrate of an efflux mechanism.[59] However, it is noted that according to the current Biowaiver guidances of WHO and EMA, *in vitro* determinations of the permeability with cell cultures are recommended as supportive data only.

Table 2
Excipients[a] Present in ATO/Proguanil IR Solid Oral Drug Products[b] With an MA in Austria (AT), Belgium (BE), Canada (CA), Switzerland (CH), Czech Republic (CZ), Germany (DE), Denmark (DK), Spain (ES), Finland (FI), France (FR), Greece (GR), Hungary (HU), Ireland (IE), Italy (IT), The Netherlands (NL), Norway (NO), Poland (PL), Portugal (PT), Sweden (SE), Slovakia (SK), The United Kingdom (UK), and The United States (US)[c], and the Minimal and Maximal Amount of that Excipient Present Pro Dosage Unit in Solid Oral Drug Products With an MA in the US[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)
Calcium carbonate	FR([1]) NL([2]) UK([3,4])	8.6-350
Cellulose, microcrystalline	AT([5,6]) BE([7-11]) CA([12-17]) CH([18,19]) CZ([20]) DE([21]) DK([22-27]) ES([28-31]) FI([32,33]) FR([34-41]) GR([42,43]) HU([44]) IE([45-48]) IT([49,50]) NL([51-55]) NO([56,57]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([64-67]) US([68-72])	4.6-1553[e]
Crospovidone	BE([7]) CA([16]) DK([23]) ES([28]) FR([37]) IE([45]) IT([50]) NL([52]) NO([57]) UK([64]) US([71])	4.4-365
Gelatin	FR([1]) NL([2]) UK([3,4])	1-657[e]
Hydroxypropylcellulose	AT([5,6]) BE([8-11]) CA([12-15,17]) CH([18,19]) CZ([20]) DE([21]) DK([22,24-27]) ES([29-31]) FI([32,33]) FR([34-36,38-41]) GR([42,43]) HU([44]) IE([46-48]) IT([49]) NL([51,53-55]) NO([56]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([65-67]) US([68-70,72])	0.2-132
Magnesium stearate	AT([5,6]) BE([7-11]) CA([12-17]) CH([18,19]) CZ([20]) DE([21]) DK([22-27]) ES([28-31]) FI([32,33]) FR([1,34-41]) GR([42,43]) HU([44]) IE([45-48]) IT([49,50]) NL([2,51-55]) NO([56,57]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([3,4,64-67]) US([68-72])	0.15-79
Poloxamers	AT([5,6]) BE([7-11]) CA([12-17]) CH([18,19]) CZ([20]) DE([21]) DK([22-27]) ES([28-31]) FI([32,33]) FR([34-41]) GR([42,43]) HU([44]) IE([45-48]) IT([49,50]) NL([51-55]) NO([56,57]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([64-67]) US([68-72])	0.9-110
Povidone	AT([5,6]) BE([7-11]) CA([12-17]) CH([18,19]) CZ([20]) DE([21]) DK([22-27]) ES([28-31]) FI([32,33]) FR([34-41]) GR([42,43]) HU([44]) IE([45-48]) IT([49,50]) NL([51-55]) NO([56,57]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([64-67]) US([68-72])	0.17-240
Silica	AT([5]) BE([8,11]) CA([12]) CH([18]) DK([22,25-27]) ES([29]) FI([33]) FR([36,38,40,41]) IE([46,48]) NL([51,53,54]) SE([60,62]) UK([66,67]) US([69,70])	0.1-100
Sodium starch glycolate	AT([5,6]) BE([8-11]) CA([12-15,17]) CH([18,19]) CZ([20]) DE([21]) DK([22,24-27]) ES([29-31]) FI([32,33]) FR([34-36,38-41]) GR([42,43]) HU([44]) IE([46-48]) IT([49]) NL([51,53-55]) NO([56]) PL([58]) PT([59]) SE([60-62]) SK([63]) UK([65-67]) US([68,69,72])	2-876[e]
Starch	FR([1]) NL([2]) UK([3,4])	0.44-616[e]

1. PALUDRINE 100 mg cp séc. 2. Paludrine 100 mg tabletten. 3. Paludrine 100 mg tablets. 4. Paludrine/Avloclor Anti-malarial Travel Pack (Chloroquine and Proguanil Anti-malarial Tablets). 5. Atovaquon/Proguanilhydrochlorid STADA 250 mg/100 mg Filmtabletten. 6. Malarone 250 mg/100 mg Filmtabletten. 7. Atovaquone/Proguanil Mylan 250 mg/100 mg filmomhulde tabletten. 8. Atovaquone/Proguanil Teva 62.5 mg/25 mg, -250 mg/100 mg filmomhulde tabletten. 9. Malarone Junior 62.5 mg/25 mg filmomhulde tabletten. 10. MALARONE 250 mg/100 mg filmomhulde tabletten. 11. PROVAQUONEG 250 mg/100 mg filmomhulde tabletten. 12. [Pr]Atovaquone and Proguanil Hydrochloride (250 mg Atovaquone/100 mg Proguanil Hydrochloride Tablets) [Glenmark Pharmaceuticals Canada Inc.]. 13. [Pr]ATOVAQUONE PROGUANIL (250 mg Atovaquone + 100 mg Proguanil Hydrochloride) Tablets [Sanis Health Inc.]. 14. [Pr]MALARONE® (250 mg Atovaquone + 100 mg Proguanil Hydrochloride) Tablets. 15. [Pr]MALARONE® PEDIATRIC (62.5 mg Atovaquone + 25 mg Proguanil Hydrochloride) Tablets. 16. [Pr]MYLAN-ATOVAQUONE/PROGUANIL (250 mg Atovaquone + 100 mg Proguanil Hydrochloride) Tablets. 17. [Pr]TEVA-ATOVAQUONE PROGUANIL (250 mg Atovaquone + 100 mg Proguanil Hydrochloride) Tablets. 18. Atovaquon Plus Spirig HC® Filmtabletten 250/100. 19. Malarone®/Junior Filmtabletten 250/100 mg, 62.5/25 mg. 20. Malarone, potahované tablety 250 mg/100 mg. 21. Malarone® Junior/Malarone® Filmtabletten 62.5/25 mg, 250/100 mg. 22. Atovaquone/Proguanil "ratiopharm", filmovertrukne tabletter (250/100 mg). 23. Provaqomyl, filmovertrukne tabletter (250/100 mg). 24. Malarone, filmovertrukne tabletter (62.5/25 mg, 250/100 mg). 25. Malastad, filmovertrukne tabletter (250/100 mg). 26. Atovaquon/Proguanilhydrochlorid "Stada," filmovertrukne tabletter (250/100 mg). 27. Atovaquon/Proguanilhydrochlorid "Glenmark", filmovertrukne tabletter (250/100 mg). 28. Atovacuona/Hidrocloruro de proguanil Mylan 250 mg/100 mg comprimidos recubiertos con película EFG. 29. Atovacuona/Hidrocloruro de proguanil Viso Farmacéutica 250 mg/100 mg comprimidos recubiertos con película EFG. 30. Malarone 250 mg/100 mg comprimidos recubiertos con película. 31. Malarone Pediátrico 62.5 mg/25 mg comprimidos recubiertos con película. 32. Malarone 250 mg/100 mg tabletti, kalvopäällysteinen. 33. Rumbabor 250 mg/100 mg, 62.5 mg/25 mg tabletti, kalvopäällysteinen. 34. MALARONE 250 mg/100 mg cp pellic, 62.5 mg/25 mg cp pellic enfant. 35. ATOVAQUONE/PROGUANIL BIOGARAN 250 mg/100 mg cp pellic, 62.5 mg/25 mg cp pellic enfant. 36. ATOVAQUONE/PROGUANIL EG 250 mg/100 mg cp pellic. 37. ATOVAQUONE/PROGUANIL MYLAN 250 mg/100 mg cp pellic. 38. ATOVAQUONE/PROGUANIL SIGMA-TAU 250 mg/100 mg cp pellic. 39. ATOVAQUONE/PROGUANIL ZENTIVA 250 mg/100 mg cp pellic, 62.5 mg/25 mg cp pellic enfant. 40. ATOVAQUONE/PROGUANIL SANDOZ 250 mg/100 mg cp pellic, 62.5 mg/25 mg cp pellic enfant. 41. ATOVAQUONE/PROGUANIL TEVA 250 mg/100 mg cp pellic, 62.5 mg/25 mg cp pellic enfant. 42. Malarone επικαλυμμένα με λεπτό υμένιο δισκία 250 mg/100 mg. 43. Malarone Παιδιατρικό Δισκίο (62.5 mg/25 mg). 44. Malarone 250 mg/100 mg filmtabletta. 45. Atovaquone/Proguanil Hydrochloride 250 mg/100 mg film-coated tablets [Generics (UK) Ltd.]. 46. Atovaquone/Proguanil Hydrochloride 250 mg/100 mg film-coated tablets [Glenmark Pharmaceuticals Europe Ltd.]. 47. Malarone 250 mg/100 mg film-coated tablets. 48. Malusone 250 mg/100 mg Film-coated tablets. 49. MALARONE 250 mg/100 mg/-Bambini 62.5 mg/25 mg compresse rivestite con film. 50. Atovaquone e Proguanile Mylan Generics 250 mg/100 mg compresse rivestite con film. 51. Atovaquon/Proguanil HCl CF 250/100 mg, filmomhulde tabletten. 52. Atovaquon/Proguanil HCl Mylan 250/100 mg, filmomhulde tabletten. 53. Atovaquon/Proguanil HCl Teva 250 mg/100 mg, 62.5 mg/25 mg filmomhulde tabletten. 54. Atovaquon/Proguanilhydrochloride Glenmark 250 mg/100 mg Filmomhulde Tabletten. 55. Malarone 250/100 mg, Junior 62.5/25 mg filmomhulde tabletten. 56. Malarone 250 mg/100 mg, Junior 62.5 mg/25 mg filmdrasjerte tabletter. 57. Provaqomyl 250 mg/100 mg filmdrasjerte tabletter. 58. Malarone, 250 mg + 100 mg, tabletki powlekane. 59. Malarone 250 mg/100 mg comprimidos revestidos por película. 60. Atovakvon/Proguanil Glenmark 250 mg/100 mg filmdragerade tabletter. 61. Malarone 250 mg/100 mg, Junior 62.5/25 mg filmdragerade tabletter. 62. Malastad 250 mg/100 mg filmdragerade tabletter. 63. Malarone 250 mg/100 mg filmom obalené tablety. 64. Atovaquone/Proguanil Hydrochloride 250 mg/100 mg Film-coated tablets [Generics UK T/A Mylan]. 65. Malarone® 250 mg/100 mg, paediatric 62.5 mg/25 mg film-coated tablets. 66. Maloff Protect 250 mg/100 mg tablets. 67. Reprapog 250 mg/100 mg, 62.5 mg/25 mg Film-coated Tablets. 68. ATOVAQUONE AND PROGUANIL HCL atovaquone and proguanil hydrochloride tablet, film coated [Prasco Laboratories]. 69. Atovaquone and proguanil hydrochloride tablet, pediatric tablet, film coated (250/100 mg, 62.5/25 mg)[Glenmark Pharmaceuticals Inc.]. 70. Atovaquone and proguanil hydrochloride tablet, film coated (250/100 mg) [BluePoint Laboratories]. 71. Atovaquone and proguanil hydrochloride tablet, film coated (250/100 mg, 62.5/25 mg) [Mylan Pharmaceuticals Inc.]. 72. MALARONE—atovaquone and proguanil hydrochloride tablet, film coated (250/100 mg, 62.5/25 mg).

[a] Substances are excluded if it can be assumed that the constituents are only present in the coating/polish or are used as printing inks.

[b] Excluded are combination products containing proguanil hydrochloride and chloroquine phosphate in 1 tablet/dosage unit.

[c] Sources of data: AT, https://aspregister.basg.gv.at/(accessed 19-9-2017); BE, www.bcfi.be/(accessed 6-9-2017); CA, https://www.canada.ca/(accessed 18-10-2017); CH, www.kompendium.ch/ (accessed 19-9-2017); CZ, www.sukl.cz/(accessed 19-9-2017); DE, www.rote-liste.de/ (accessed 19-9-2017); DK, https://laegemiddelstyrelsen.dk/ (accessed 19-9-2017); ES, www.aemps.es/ (accessed 19-9-2017); FI, www.fimea.fi/ (accessed 17-10-2017); FR, https://www.vidal.fr/(accessed 17-10-2017); GR, www.eof.gr/ (accessed 17-10-2017); HU, www.ogyi.hu/ (accessed 18-10-2017); IE, www.imb.ie/(accessed 18-10-2017); IT, https://www.torrinomedica.it/ (accessed 17-10-2017); NL, www.cbg-meb.nl. (accessed 17-10-2017); NO, www.legemiddelverket.no/(accessed 17-10-2017); PL, http://pub.rejestrymedyczne.csioz.gov.pl/ (accessed 17-10-2017); PT, http://app7.infarmed.pt/infomed/inicio.php (accessed 17-10-2017); SE, www.lakemedelsverket.se/ (accessed 17-10-2017); SK, www.sukl.sk/ (accessed 17-10-2017); UK, www.medicines.org.uk/emc/(accessed 17-10-2017); US, www.dailymed.nlm.nih.gov/. (accessed 17-10-2017).

[d] US: FDA's Inactive Ingredient Database, https://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm (version date 30-6-2017).

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

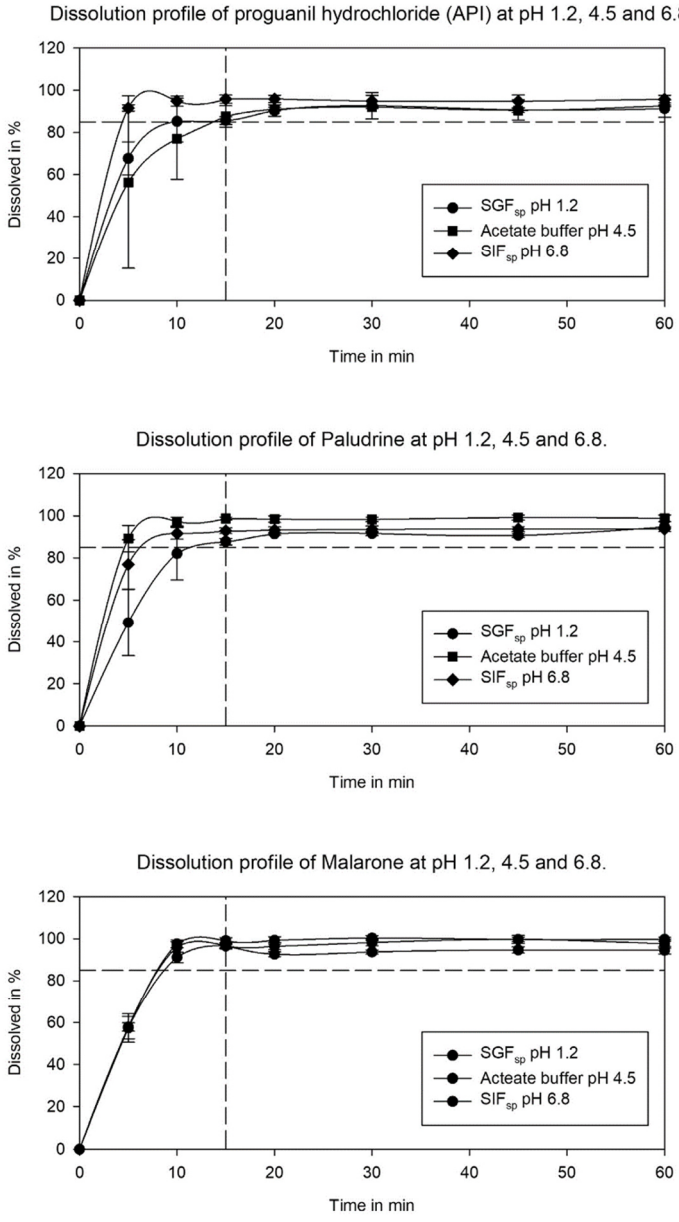

Figure 2. Dissolution profiles of proguanil hydrochloride and proguanil products at pH 1.2, 4.5, and 6.8 according to biowaiver guidelines.

Taking a conservative view, proguanil is classified as "not highly permeable" according to the BCS guidelines.

BCS Classification

On the basis of the solubility data, proguanil hydrochloride is a "highly soluble" API according to all current guidances. Because the permeability data are not conclusive for proguanil, it is conservatively categorized as "not highly permeable." Therefore, proguanil hydrochloride should currently be classified as a BCS class 3 drug. This classification is in accordance with the Biopharmaceutics Drug Disposition Classification System, which also categorized proguanil hydrochloride as a class 3 API.[69]

Dissolution

WHO, FDA, and EMA require in their guidelines "very rapid dissolution" behavior of drug substances classified to BCS class 3, which means that at least 85% of the declared amount of the API dissolves within 15 min.[5-7] For proguanil, for which the permeability studies suggest a possible efflux mechanism in the intestines, a "very rapid dissolution" is especially important. As seen in Figure 2, proguanil hydrochloride can fulfill this criterion, both as a pure drug and when formulated in commercial products.

Risk of Bioinequivalence Caused by Excipients and Manufacturing Variations

The current FDA, WHO, and EMA guidances allow for BCS class 3 biowaivers, requiring that the excipients in the test product must be qualitatively the same and quantitatively very similar to the reference product.[5-7] Excipient concerns revolve around the possibility that excipient differences may result in bioinequivalence, which cannot be detected by *in vitro* dissolution testing, for example, intraluminal drug binding, modulation of intestinal transit, intestinal drug metabolism, intestinal absorption, and permeability.

From Caco-2 cell monolayer studies, it appears that proguanil may subject to an efflux mechanism, and therefore prone to excipient effects.[59] However, it should be noted that many drugs have been shown to be efflux substrates *in vitro*, without any evidence that this translates into absorption interactions *in vivo*. For example, cimetidine has been shown to be a Pgp substrate.[70] In previous work, it was shown that even large amounts of 12 common excipients did not impact the absorption of either cimetidine or aciclovir, another BCS class 3 drug.[71] The 12 excipients were sodium lauryl sulfate, corn starch, sodium starch glycolate, colloidal silicon dioxide, dibasic calcium phosphate, crospovidone, lactose, povidone, stearic acid, pregelatinized starch, croscarmellose sodium, and magnesium stearate, many of which are present in proguanil products (see Table 2).

Structurally, proguanil is a biguanide, like the more highly studied drug metformin. The BCS characteristics of metformin have been previously discussed.[72-74] Although metformin shows reduced absorption with higher doses,[75] no excipient concerns for metformin have been identified. Since the absorption of proguanil is linear with dose,[38,53] the expectation is that, here too, excipient concerns would be minimal.

With respect to excipient effects on solubility and dissolution, since proguanil hydrochloride and its products show "very rapidly dissolving" dissolution behavior, dissolution-related problems with unusual amounts of excipients or variations in the manufacturing process would be easily revealed in dissolution studies.

Combining proguanil hydrochloride's lack of food effect[31] together with its dose-linearity, behavior of similar drugs and known BE of products containing the excipients listed in Table 2, it is reasonable to conclude that its absorption is unlikely to be influenced by common excipients used in their usual amounts. Furthermore, it should be noted that the root cause of the low permeability and incomplete absorption of proguanil appears to be the extensive protonation of the molecule.

Proguanil hydrochloride is often used in FDC products, particularly with ATO. Although the opportunity for a BCS-based biowaiver approval in this case also depends on the physical and metabolic compatibility of ATO and proguanil hydrochloride, no such incompatibilities between these 2 APIs have been reported.

Patient's Risk Associated With Bioinequivalence

Bioinequivalent products can either cause subtherapeutic or supratherapeutic drug levels in patients. Subtherapeutic levels of proguanil/cycloguanil could lead not only to a failure of therapy but also encourage the emergence of resistance. In contrast, supratherapeutic concentrations could result in adverse effects.

Despite the existing resistance to proguanil when it is used as a single therapeutic agent, the former risk seems to be low since proguanil hydrochloride is now recommended to be applied in combination with other antimalarials. In case of the latter risk, it must be considered that proguanil hydrochloride is a drug with a wide therapeutic index. It is well-tolerated, even in pregnancy, with adverse effects in clinical studies, such as headache, nausea, vomiting, and abdominal pain, being mild and observed with low frequency.[1,25,58] Undesirable effects of approved marketed products have been reported without indication of frequency and reports about severe adverse effects are rare.

Therefore, the risk associated with either subbioequivalent or suprabioequivalent products of proguanil hydrochloride appears to be low and acceptable.

Conclusion

A BCS-based biowaiver approval for IR solid oral dosage forms of proguanil hydrochloride can be recommended in accordance with the Biowaiver guidelines of the FDA, WHO, and EMA. Excipients in new generic product formulations should be well-established and included in usual amounts. In addition, both test and comparator products need to fulfill the dissolution test criteria for "very rapidly dissolving," which means a drug release greater than 85% in 15 min in media at pH 1.2, 4.5, and 6.8, as recommended in the FDA, EMA, and WHO guidances.

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Global Health Commentary

Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Cephalexin Monohydrate

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ABSTRACT

Literature data and results of experimental studies relevant to the decision to allow waiver of bioequivalence studies in humans for the approval of immediate release solid oral dosage forms containing cephalexin monohydrate are presented. Solubility studies were performed in accordance with the current biowaiver guidelines of the Food and Drug Administration, World Health Organization and European Medicines Agency, taking the degradation at some pH values into consideration. Together with solubility and permeability data for cephalexin monohydrate from the literature, it was demonstrated to be a Biopharmaceutics Classification System Class 1 drug. The pharmacokinetic behavior, results of bioequivalence studies published in the literature, as well as the therapeutic uses, potential toxicity and potential excipient effects on bioavailability were also assessed. Cephalexin has a wide therapeutic index and no bioequivalence problems have been reported. Dissolution studies were run under Biopharmaceutics Classification System–biowaiver conditions for the pure drug and 2 generic formulations available on the German market. Considering all relevant aspects, it was concluded that a biowaiver-based approval for products containing cephalexin monohydrate as the single active pharmaceutical ingredient is scientifically justified, provided that well-established excipients are used in usual amounts and that both test and reference dosage forms meet the guideline criteria of either "rapidly dissolving" or "very rapidly dissolving."

Introduction

Cephalexin was introduced in the 1960s as a semisynthetic derivative of cephalosporin C.[1] It is an orally administrable antibacterial agent of the β-lactam group and is used commercially in the hydrate form. While it is particularly effective against *Staphylococci* and *Streptococci* infections, it has a comparably narrow spectrum of efficacy. Currently, the World Health Organization (WHO) Model List of Essential Medicines (EML) lists cephalexin monohydrate as a key access antibiotic and recommends the use of solid oral dosage forms of cephalexin as the second choice antibiotic for the therapy of exacerbations of chronic obstructive pulmonary disease (COPD), pharyngitis, and skin and soft tissue infections.[2] Additional therapeutic indications are well-known and are presented in this work.

This biowaiver monograph for cephalexin monohydrate refers to drug products containing cephalexin monohydrate as the single

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active pharmaceutical ingredient (API) and is based on data concerning the clinical, pharmacokinetic and biopharmaceutical properties of cephalexin from the open pharmaceutical literature in combination with new experimental data generated to complete the drug profile. The literature data are carefully reviewed with respect to the different mandatory aspects for a biowaiver procedure, that is, the approval of generic immediate release solid oral dosage forms based on *in vitro* dissolution tests rather than on *in vivo* pharmacokinetic studies to evaluate bioequivalence (BE).

The experimental studies evaluated the solubility and degradation behavior of cephalexin as well as comparing the dissolution performance of the pure drug with commercial formulations. The risks of waiving *in vivo* bioequivalence testing for the approval of new multisource or reformulated immediate release solid oral dosage forms containing cephalexin monohydrate in consideration of potential influences of excipients and the product formulation are evaluated in the context of public health.

The aim and scope of the biowaiver monograph series have been explained before in the literature.[3] The systematic approach to recommend biowaiver approval (or not) is rooted in the guidelines from the United States Food and Drug Administration (FDA), the WHO and the European Medicines Agency (EMA).[4-6] In the context of the monograph series, almost 50 biowaiver monographs for various active pharmaceutical ingredients (APIs) have already been published. These monographs are available on an open access basis from the website of the International Pharmaceutical Federation (https://www.fip.org/bcs-monographs) as well as on the website of the journal.

Materials and Methods

Materials

Pure drug substance of cephalexin monohydrate of European Pharmacopoeia Reference Standard quality and of British Pharmacopoeia (BP)/United States Pharmacopoeia (USP) grade were used for *in vitro* studies. The European Pharmacopoeia (Ph. Eur.) Reference Standard (Batch ID 00WWN1, 0182I7, 01GC04, 013BK0, 013162 and 013XA2) was directly obtained from Council of Europe-EDQM (Strasbourg, France) while the BP/USP grade powder (Lot # QR14917) was purchased from MP Biomedicals (Solon, OH). The pure drug substances were stored at 2–8 °C. The marketed products Cephalexin-ratiopharm® 1000 mg film-coated tablets (Lot # HC4916) from Ratiopharm GmbH (Ulm, Germany) and Cephalex-CT 1000 mg (Lot # GX9912) from AbZ-Pharma GmbH (Ulm, Germany) were purchased from Phoenix Pharmahandel GmbH&Co. KG (Hanau, Germany), a German pharmaceutical wholesaler. Ammonium acetate, potassium chloride, and sodium dihydrogen phosphate dihydrate were purchased from Merck KGaA (Darmstadt, Germany). Potassium phosphate monobasic and potassium phthalate were purchased from Sigma-Aldrich (Steinheim, Germany) while sodium chloride was obtained from VWR International (Fontenay-sous-Bois, France). Acetic acid, ammonia, hydrochloric acid, orthophosphoric acid, and sodium hydroxide (liquid and solid) were purchased from VWR International (Fontenay-Sous-Bois, France). Acetonitrile and methanol were obtained commercially from Merck KGaA.

Methods

Literature Research

The main sources for the literature research were PubMed and Google Scholar. Standard pharmaceutical literature as well as the online service of ROTE LISTE® (German online pharmaceutical database)[7] were also used as references. Both alternative forms of spelling of cephalexin (cephalexin and cefalexin) were used in combination with the following keywords for the literature search (last updated on June 16, 2019): monohydrate, absorption, adverse effects, bioavailability, bioequivalence, crystallinity, degradation, dissolution, distribution, elimination, excipients, hygroscopicity, interaction, metabolism, permeability, salts, solubility, solvates, therapeutic index, toxicity. Relevant data regarding the general characteristics of cephalexin monohydrate, its physicochemical and pharmacokinetic properties, publicly accessible bioequivalence results, and information about different available dosage forms and their excipients were collected.

Solubility and Degradation Studies

The solubility of cephalexin monohydrate was determined in accordance with the biowaiver guidances of the FDA, the WHO and the EMA. The solubility was evaluated with a modified shake-flask method at different pH values over a range of 1–6.8, including at the pK_a value of the substance and at pH values one unit below and above the pK_a. All determinations were performed in triplicate.

To determine whether the "highly soluble" criterion according to the BCS (dose/solubility ratio (D/S) ≤250 mL) is fulfilled for cephalexin, both the highest dosage strength and the highest single therapeutic dose were considered. In the case of cephalexin monohydrate the highest dosage strength and the highest single therapeutic dose are the same and equivalent to 1000 mg of the monohydrate. Since the modified shake-flask method only requires a test volume of 3 mL, the amount of cephalexin added was downscaled accordingly. Compendial buffers from the United States Pharmacopoeia (USP) and the European Pharmacopoeia (Ph. Eur.) were chosen as the test media. The following buffers were prepared: potassium chloride/hydrochloric acid solutions at pH 1.0 and 1.2 (according to USP),[8] a potassium chloride/hydrochloric acid buffer at pH 1.7 (USP, pH corresponding to 1 unit below the pK_a),[8] an adjusted phosphate buffer at pH 2.7 (Ph. Eur., corresponding to the pK_a value of cephalexin),[9] an adjusted phthalate buffer at pH 3.7 (Ph. Eur., pH corresponding to 1 unit above the pK_a),[9] an acetate buffer at pH 4.5 (Ph. Eur.),[9] and a phosphate buffer at pH 6.8 (USP).[8] The pH of each buffer was adjusted to ±0.05 units of the specified value and recorded before the solubility study. An amount of ~13 mg cephalexin monohydrate was accurately weighed into each of 21 Uniprep™ vials (Whatman™, Little Chalfont, UK). Three milliliters of the buffer of interest was added to each of 3 vials, and this was repeated for each of the 7 buffers. Thus, if the entire amount (13 mg) of cephalexin added to the vial dissolves in the 3 mL volume of buffer, one can conclude that cephalexin complies with the D/S ratio requirement for "highly soluble" (4 mg/mL = 1000 mg/250 mL) in the given buffer. In the first series of experiments, the vials were shaken in an orbital shaker (Heidolph POLYMAX 1040, Schwabach, Germany) at 37 ± 0.5 °C for 24 hours, following which the samples were filtered through the polytetrafluorethylene (PTFE) membrane (pore size: 0.45 μm) integrated in the Uniprep™ vials, an aliquot from each sample was removed and appropriately diluted for analysis, and the pH value was measured and recorded.

Since degradation of cephalexin monohydrate was expected at pH 6.8 and observed at pH 6.8 and 4.5 in form of a change in color and odor of the samples, accompanied by changes in the high liquid performance (HPLC) chromatograms, a degradation study was performed at these pH values. For this purpose, clear solutions of cephalexin monohydrate in both of the buffers at a concentration of at least 4 mg/mL were prepared and filtered through a PTFE membrane with a pore size of 0.45 μm. After shaking at 37 °C, the samples were drawn at 60, 120, 180, and 240 minutes, with a control sample also removed prior to shaking at 37 °C. The samples were immediately diluted and analyzed by HPLC. Based on the results of the degradation experiments and on literature recommendations, 3 hours was chosen as an appropriate time period for the subsequent

solubility studies, which were performed at both pH 4.5 and 6.8. Except for the time of incubation, the setup of the solubility experiments at pH 4.5 and 6.8 remained as described above.

In Vitro *Dissolution Studies*

The dissolution performance of 1000 mg cephalexin monohydrate as the pure active pharmaceutical ingredient and in 2 commercial products was evaluated with an Erweka® Dissolution Tester (USP Apparatus II, DT80, Erweka GmbH, Heusenstamm, Germany) at pH 1.2, 4.5 and 6.8 at 37 ± 0.5 °C, with each test conducted in triplicate. Dissolution tests with the pure drug were performed in order to detect potential effects of the product formulation on the dissolution performance of the substance. The API powder was encapsulated in 2 gelatine capsules (size 000, Club Vits Ltd., East Yorkshire, UK) per dose and these were inserted in sinkers to reduce floating of the substance on the dissolution medium. The cephalexin products, Cephalex-CT and Cephalexin-ratiopharm® (both film-coated tablets), are 2 generic products which are available on the German market. Since the biowaiver guidelines require slightly different dissolution media, volumes and agitation speeds for *in vitro*-dissolution tests,[4-6] the most widely used conditions were adopted for the dissolution studies. The lowest volume (500 mL) was chosen to better reflect the physiological liquid volume in the upper gastrointestinal tract in the fasted state. Standard USP buffers with buffer capacities similar to those of buffers from the other 2 pharmacopoeias were chosen as dissolution media,[10] that is, simulated gastric fluid without pepsin (SGF_{sp}, USP)[8] with a pH of 1.2, an acetate buffer at pH 4.5 (Ph. Eur.)[9] and simulated intestinal fluid without enzymes (SIF_{sp}, USP)[8] at a pH of 6.8. They were prepared and degassed according to the instructions of the USP for dissolution media.[11]

pH 1.2: Dissolution tests in SGF_{sp} pH 1.2 were run in a volume of 500 mL at 50 rpm and 37 °C. Sampling times were after 5, 10, 15, 20, 30, 45 and 60 minutes. Samples of 1 mL were withdrawn from the vessels and replaced with 1 mL of fresh, pre-warmed medium. The samples were filtered through a Whatman® PTFE syringe filter 0.45 μm (Whatman GmbH, Dassel, Germany) and diluted for quantification. The temperature and the pH of the medium were measured before and after the experiment and all values were recorded.

pH 4.5 and 6.8: Preliminary dissolution tests of the film-coated tablets at pH 4.5 and 6.8 showed degradation of dissolved drug during the test and also during the period between sampling and analysis. Therefore, the tests were run sequentially, vessel for vessel, to minimize the time between sampling and HPLC analysis. The samples, which were taken at 15 and 30 minutes from each vessel, were filtered through a Whatman® PTFE syringe filter 0.45 μm (Whatman GmbH, Dassel, Germany), diluted and analyzed immediately via HPLC. That procedure was established referring to another biowaiver monograph, acetylsalicylic acid, which describes a modified dissolution method for the prevention of degradation issues.[12] Visual inspection of preliminary studies, especially at pH 4.5, revealed high variations in the disintegration behavior of the tablets and coning, so additional tests were conducted at 75 rpm in 900 mL for these 2 media. As for pH 1.2, the temperature and the pH of the medium were measured before and after the experiment and all values were recorded.

HPLC Analysis

The concentration of dissolved cephalexin monohydrate in all diluted samples from solubility, degradation and dissolution studies was analyzed by HPLC with ultraviolet (UV) detection. The HPLC method was based on the assay method of the European Pharmacopoeia for cephalexin monohydrate. The HPLC system consisted of a Hitachi LaChrom Elite® pump (L-2130), an autosampler (L-2200) and an UV-detector (L-2400). A LiChrospher® 100 RP-18 (5 μm) LiChroCART® 125 × 4 cartridge (Merck Milipore, Darmstadt, Germany) was used for analysis. The mobile phase consisted of methanol, acetonitrile, monobasic potassium phosphate solution (13.6 g/l, pH ~4.5) and water in a ratio of 2:5:10:83. The flow rate was set at 1.5 mL/min, the injection volume at 20 μL and the detection wavelength at 254 nm. The run time was 10 minutes and the retention time approximately 5 min. The method was validated with respect to linearity and range, precision, detection limit (~0.4 μg/mL) and quantitation limit (~1.2 μg/mL) in accordance with the International Conference on Harmonization (ICH) guideline Q2(R1).[13]

General Characteristics

Name and Structure

Cephalexin monohydrate can also be found in the literature, including the European Pharmacopoeia, under an alternative spelling, that is, cefalexin monohydrate.[14] The International Nonproprietary Name proposed by the WHO is cephalexin while the Modified International Nonproprietary Name (INN) is cephalexin monohydrate.[15,16] Chemical names differ dependent on the source for example (6R,7R)-7-[[(2R)-2-Amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid-monohydrate (IUPAC, Ph. Eur.),[14] (6R,7R)-7-[(R)-2-Amino-2-phenylacetamido]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid monohydrate (USP),[17] D-7-(2-Amino-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4,2,0]oct-2-ene-2-carboxylic acid monohydrate.[15,16] Other variations can be found in the pharmaceutical literature.[18,19]

The molecular formula of cephalexin monohydrate is $C_{16}H_{17}N_3O_4S \cdot H_2O$.[14] The corresponding molecular weight is 365.4 g/mol.[14] Figure 1 depicts the structure of cephalexin monohydrate.

The CAS number of cephalexin monohydrate is 233-25-78-2[20] in contrast to 15686-71-2 (anhydrous substance),[20] 66905-57-5 (dihydrate),[20] 105879-42-3 (hydrochloride)[20] and 53950-14-4 (lysine).[21]

Therapeutic Indication and Dosing

Cephalexin is a β-lactam antibacterial, specifically a first generation, first group acid-stable oral glycyl-cephalosporin.[18,19,22] Like all β-lactam antibiotics, it competitively inhibits the transpeptidase in the last step of the peptidoglycan synthesis during biosynthesis of the bacterial cell wall.[23] As a consequence, the bacterial cell walls cannot resist osmotic-dependent bacteriolysis.[21,23] The bactericidal effect of cephalexin is thus limited to growing bacteria only.[19] In general, cephalexin can be effective against both Gram-positive and Gram-negative microorganisms,[24,25] although it is most effective against different strains and isolates of *Streptococci* and *Staphylococci*.[22,23] Many *in vitro* susceptibility results, which were often simultaneously measured with plasma levels in clinical studies conducted in the years following the introduction of cephalexin,

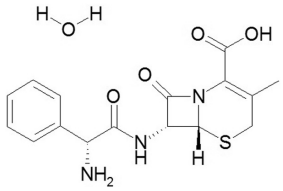

Figure 1. Structure of cephalexin monohydrate.

can be found in the open pharmaceutical literature.[26-33] Since resistance development can be an issue in antibacterial therapy, information about susceptibility/resistance needs to be kept up to date. According to the prescribing information of the marketed product Keflex® (last revision: October 2015) and of Cephalex-CT® (February 2018, data from national resistance monitoring projects), the following aerobic Gram-positive microorganisms are considered to be susceptible to cephalexin: methicillin-susceptible isolates of *Staphylococcus aureus*,[34,35] penicillin-susceptible isolates of *Streptococcus pneumoniae*,[34] *Streptococcus pyogenes*,[34,35] *Staphylococcus saprophyticus*[35] and *Streptococcus agalactiae*.[35] Gram-negative susceptible bacteria are *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*.[34] Naturally resistant bacterial strains include the aerobic Gram-positive *Enterococcus spp.*,[34,35] methicillin-resistant *Staphylococcus aureus*,[34,35] penicillin-resistant *Streptococcus pneumoniae* (cross resistance),[34,35] the aerobic Gram-negative *Acinetobacter calcoaceticus*,[34] *Enterobacter ssp.*,[34,35] *Legionella pneumophila*,[35] *Morganella morganii*,[34] *Proteus vulgaris*,[34] *Pseudomonas ssp.*,[34,35] and various *Chlamydia* and *Mycoplasma* strains.[35] The susceptibility of *Haemophilus influenza* and *Moraxella catarrhalis* is currently the subject of controversial discussion.[34,35] With the exception of *Streptococcus pyogenes*, acquired antibiotic resistance is reported for all of the above-mentioned species.[35] Various mechanisms of resistance are known, e.g. inactivation by beta-lactamases, reduced affinity of the target proteins to cephalexin, insufficient penetration of cephalexin through the outer structure of the cell wall of Gram-negative bacteria, and transport via efflux pumps.[35]

As a matter of principle, cephalexin should only be given as treatment against susceptible microorganisms. The use of cephalexin is indicated for the treatment of infections of the skin and skin structures (caused by isolates *Staphylococcus aureus* and *Streptococcus pyogenes*), the respiratory tract (*Streptococcus pneumoniae* and *pyogenes*), the bones (*Staphylococcus aureus*, *Proteus mirabilis*) and the genitourinary tract (*Escherichia coli*, *Proteus mirabilis*, and *Klebsiella pneumoniae*), as well as for the treatment of ear-nose-throat infections, especially otitis media caused by susceptible strains of *Streptococcus pneumoniae*, *Haemophilus influenza*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Moraxella catarrhalis*.[34,35] In case of severe infections, a treatment with parenteral cephalosporins is recommended.[18]

In terms of solid oral dosage forms of cephalexin, different dosing regimens are available depending on the severity of the infection. Usual dosing for adults and children above 12 years is 1–2 g daily divided in doses given every 6, 8, or 12 h (e.g., 250 mg every 6 h or 500 mg every 6, 8, or 12 h).[18,34,36] Doses of 3–4 g daily in 2 to 4 divided doses can also be administered (e.g., 1000 mg every 6 or 8 h).[34-36] An increase to a maximum daily dose of 6 g is possible as an alternative to parentally applied cephalosporin.[18] Pediatric patients may be treated with 25–100 mg/kg, 50–100 mg/kg (in case of severe infections) and 75–100 mg/kg (in the case of otitis media), respectively.[18,34] In these patients, a maximum daily dose of 4 g should not be exceeded.[18] Treatment is usually prescribed to extend over 7, 10, or 14 days or in some cases until 2 to 3 days after remission of the symptoms.[34-36] With respect to infections of β-hemolytic streptococci (*Streptococcus pyogenes*), a duration of at least 10 days is suggested to circumvent long-term complications.

Therapeutic Index and Toxicity

Toxicity and Adverse Effects in Animals

A median lethal dose (LD_{50}) value of 1600–4500 mg/kg body weight has been established in mice, while in rats it is >5.0 g/kg (lower values for weanlings and newborns), in cats >0.5 g/kg, in dogs >0.5 g/kg and in monkeys >1.0 g/kg body weight.[35,37] Adverse effects observed during toxicity studies included diuresis and polyuria, dehydration, ptosis, lethargy and anorexia (observed in mice at doses of 2–4.5 g/kg body weight), hypothermia and tremor. Intraperitoneal LD_{50} values have also been published for mice (0.4–1.3 g/kg body weight), rats (>3.7 g/kg), rabbits (>4.0 g/kg), cats (>1.0 g/kg) and dogs (>1.0 g/kg).[37] At doses more relevant to those administered to humans, cephalexin monohydrate showed only minor toxicity when administered orally as single doses to mice, rats, dogs, cats or monkeys.

The chronic toxicity of orally administered cephalexin has also been evaluated in several studies. Long-term studies with rats which received up to 675 mg/kg body weight daily did not exhibit toxic side effects regarding hematology, blood chemistry and histopathology of the organs.[35] Another study of chronic toxicity with 20 young rats that received 250, 500, or 1000 mg of cephalexin per kg body weight daily showed the same lack of toxicity after 380 days of treatment. Doses of 1000 mg per kg for rats and 400 mg per kg for dogs for 1 month and 1 year, respectively, and doses of 400 mg/kg for monkeys for one year were tolerated without severe events.[37] In rats, transient mild diarrhea, transitory growth suppression and enlargement of the cecum and colon were detected. Dogs showed transient appetite suppression, salivation, emesis and temporarily diarrhea, while in monkeys, only salivation and mild diarrhea were observed.

In rabbits treated repeatedly with intraperitoneal doses of 4 g/kg, changes in the composition of the fecal flora were observed after diarrhea. In other animal species, vacuolar nephrosis was detected in renal tissue. Intravenous doses for 14 days were well-tolerated by rats (15–60 mg/kg) and dogs (7.5–30 mg/kg) without the occurrence of adverse effects.

Toxicity and Adverse Effects in Humans

The most common adverse reactions to cephalexin in humans are related to the gastrointestinal tract.[34] Diarrhea, which is the most frequent adverse effect reported in clinical trials, has been observed along with nausea, emesis, a lack of appetite, abdominal discomfort and pain, dyspepsia, meteorism, gastritis and colitis.[34-36] Most of these side effects are mild and reversible even during therapy.[35,36] Hematological effects observed in clinical trials include thrombocytopenia, neutropenia, leukopenia and eosinophilia, all of which are reversible (reported without information on frequency),[35,36] pancytopenia, hemolytic anemia, aplastic anemia, agranulocytosis, and hemorrhage.[34] Hepatic effects, including slight and reversible elevations in serum glutamic oxaloacetic transaminase and serum glutamate pyruvate transaminase, slight elevations in alanine transaminase, aspartate transaminase, alkaline phosphatase, and bilirubin values, transient hepatitis and cholestatic jaundice have also been reported in clinical trials.[34-36] Renal effects reported include interstitial nephritis, renal dysfunction, and toxic nephropathy. Headache, dizziness, fatigue, confusion and even agitation and hallucinations have been recorded in clinical trials, as have hypersensitivity reactions such as rash with pruritus and swelling (Quincke's edema, joint swelling), urticaria, angioedema, erythema multiforme, Stevens-Johnson or Lyell syndrome, acute exanthemic generalized pustulosis, toxic epidermal necrolysis, furthermore genital and anal pruritus, candidiasis, vaginitis and vaginal discharge, arthralgia, arthritis, joint disorder, and fever. Certain laboratory parameters like blood urea nitrogen, creatinine, and lactate dehydrogenase can also be increased.[34] In addition, laboratory tests, for example tests for glucosuria or the Coomb's test, can show false positive results during therapy with cephalexin.[34,35]

All hypersensitivity reactions are considered to be serious adverse effects,[34-36] as they can culminate in anaphylaxis. 2–3% of

patients who are not sensitive to penicillin react allergically to cephalexin.[35] A cross-hypersensitivity among β-lactam antibiotics results in a larger risk of hypersensitivity, around 8-12%[34,35] of the patients with an allergic reaction to penicillin in the past developed allergic symptoms to cephalexin. Therefore, a meticulous record of the patient's medical history is crucial. In the case of a hypersensitivity reaction, the medication should be stopped immediately and appropriate therapy should be started. This applies also in terms of *Clostridium difficile*-associated diarrhea and pseudomembranous colitis,[34-36] which may occur during any antibiotic treatment and are also serious complications. Other severe undesirable effects that require discontinuation of therapy include a positive direct Coomb's-test with acute intravascular hemolysis, or, if seizures occur, as these can be caused by cephalexin especially in patients with decreased renal function.[34]

Patients with renal or hepatic impairment or who are receiving long-term therapy can show a prolonged prothrombin time and must be monitored carefully. An adjusted dose regimen must be applied for patients with renal insufficiency since renal impairment increases plasma levels and prolongs the residence time of cephalexin in the body (as explained in the paragraph "Absorption and Bioavailability" below).[34-36] In general, the maximum daily dose is reduced for adults and children above 12 or 15 years old if the creatinine clearance is below 50−60 mL/min, while the dosing interval should be extended if it is below 30 mL/min.

Consideration should also be given to the development of drug resistance. The use of cephalexin for infections mainly caused by non-susceptible microorganisms or the long-term or repeated application of cephalexin can lead to an overgrowth of non-susceptible organisms and a superinfection (e.g., *moniliasis vaginalis*).[34-36] In terms of public health, the appearance of cephalexin-resistant bacteria could also be a consequence of using cephalexin to treat non-susceptible organisms.

Overdosage with cephalexin can result in nausea, vomiting, diarrhea, gastrointestinal cramps and epigastric distress, hematuria, cerebral disorders and, in the case of hypersensitivity to cephalexin, anaphylactic reactions.[34-36] Measures other than to discontinue the medication and to take general supportive actions depending on the symptoms are not necessary. In cases of 5-fold or more overdoses, gastric lavage is recommended.[36]

In general, cephalexin toxicity appears to be minor, with the exception of allergic reaction. Cephalexin does not appear on the FDA Guidance for Scale-Up and Post-Approval Changes list for narrow therapeutic index (NTI) drugs.[38] Additionally, since animal experiments with 30 fold higher single doses than daily doses in therapeutic regimen for humans showed no distinct adverse effects, it would not be classified as a narrow therapeutic index drug according to the FDA definitions.[32,39] Underlining these remarks, Griffith et al. reported no significant changes in hematologic, hepatic or urinary status of healthy volunteers who received double the daily dose of cephalexin for 2 weeks.[32] Besides, most of the adverse reactions are tolerable and the few severe adverse effects that do occur can be prevented by obtaining a full medical history, monitoring the patient and prescribing the correct therapy.

Physicochemical Properties

Salts, Hydrates and Solvates, Stereoisomers

The European Pharmacopoeia lists cephalexin as cefalexin monohydrate.[14] In the monographs of the United States Pharmacopoeia it is described as cephalexin, which can be the anhydrous substance or the monohydrate[17] and as cephalexin hydrochloride, which is also a monohydrate.[40] The International Pharmacopoeia does not include a cephalexin monograph. Other salts reported in the literature include cephalexin benzathine, lysine or sodium.[21]

Several solvated forms and in addition isomorphic anhydrides of cephalexin have been reported (pseudopolymorphism): anhydrous cephalexin, (desolvated) monohydrate, cephalexin dihydrate, (hydrated) demethanolate, and (hydrated) deacetonitrilate,[41-43] all of which show different hygroscopicities and water adsorption characteristics.[44,45] The most stable form is the monohydrate, into which the anhydrous form is transformed when the relative humidity is above 20%, or when the dihydrate is stored at a relative humidity of <62%.[45] The transformation of the monohydrate into the dihydrate form is observable at relative humidities greater than 70%.[42]

Cephalexin can also exist in an amorphous form (with different physicochemical characteristics) that does not recrystallize between 20 and 35 °C when the relative humidity is below 66%.[46,47] Griffith et al. reported a different absorption behavior and reduced blood levels for the anhydrous form.[32] Even in the amorphous state, differently hydrated forms with specific water adsorption behavior were identified depending on the exact storage conditions (monohydrate and dihydrate).[41,44] Amorphous cephalexin can be prepared by freeze-drying or by milling for more than 4 h,[46,48] although some of the properties of the material e.g. water adsorption/content, dehydration and decomposition temperature and solubility, may differ according to the process used.[44,49]

In addition to the crystalline or amorphous forms, cephalexin powders with an intermediate crystallinity have been identified.[48] These can be generated by recrystallization processes from non-crystalline cephalexin, grinding or tabletting.[46,48,50] For example, Otsuka and Kaneniwa demonstrated that 10 min of grinding reduced the crystallinity of cephalexin to 20%.[48] The physicochemical properties such as water content, dehydration and decomposition point and surface area changed depending on the process parameters (e.g., grinding time) and on the resulting crystallinity (e.g., powders with a crystallinity below 25% showed rapidly changing physicochemical characteristics).[49] Therefore, the influence of environmental parameters such as humidity, temperature or mechanical stress during the manufacturing process, on the powder characteristics must be considered.

The stereochemical form of cephalexin monohydrate has a large influence on the absorption process and the antibacterial effect of the drug. Caco-2 cell experiments performed by Dantzig and Bergin suggested that uptake of cephalexin occurs by energy- and proton-dependent peptide transporters which have a higher affinity for the L-enantiomer of cephalexin.[51] Thwaites and Daniel confirmed these results in further Caco-2 cell studies.[52]

Snyder et al. reported that structural isomers such as a changed C7 bond or an altered double bond (delta-3) had no positive effect on the uptake via the dipeptide transporter in Caco-2 cell culture studies.[53] According to their results, the antibacterial activity of both the L-stereoisomer and the above-mentioned structural isomers was dramatically reduced.[53] Prior to those experiments, *in vitro* and *in vivo* studies in rats conducted by Tamai et al. had indicated that the L-enantiomer has not only a greater affinity to the transport carrier but also to hydrolyzing enzymes located at the luminal site of enterocytes, in the cells and their basolateral membrane and in serum and urine and would therefore be hydrolyzed immediately after or even during its absorption.[54] The relevance of these studies for therapy in humans has not been established.

Isoelectric Point and pK_a

Standard pharmaceutical literature, for example Clarke's, reports pK_a values of 2.5, 5.2 and 7.3 without describing the corresponding functional groups.[19,21,55] Others have reported values of 5.2 and 7.3 (5.3 = carboxyl group, 7.1 = amino group), although

these seem to have been determined in 66% DMF.[20] pK_a values have also been determined in aqueous media using potentiometry or capillary zone electrophoresis (CZE). Yamana et al. obtained values of 2.56 and 6.88 by potentiometric titration in water with an ionic strength of 0.5 at 35°C.[56] Mrestani et al. reported results obtained with CZE and potentiometric experiments, with pKa values of 3.11 ± 0.16 and 6.79 ± 0.27 (CZE, field strength 30 kV, 25°C) and 2.34 ± 0.09 and 7.08 ± 0.06 (potentiometry, average ionic strength 0.158 M, 25.1°C), respectively.[57] A study from 2007 determined similar values with capillary electrophoresis (2.93 ± 0.11 for the carboxyl group and 7.18 ± 0.07 for the amino group, voltage 25 kV, 25°C) and potentiometric titration (2.53 ± 0.01 and 7.13 ± 0.01, ionic strength 0.2 M, 25 ± 0.1°C)[58] (The publication did not indicate the size of n and if SD or SE were reported). Cephalexin is an amphoteric substance, with 1 study reporting an isoelectric point of 4.67.[59] Others sources report different values for the isoelectric points, calculated on the basis of the dissociation constants evaluated in the respective study.[56,60,61]

Partition and Distribution Coefficient

Clarke's Analysis of Drug and Poisons reports an octanol/water coefficient (log P) for cephalexin of 0.6, which is similar to values from other sources (Drug Bank: prediction with ALOGPS program: 0.55; Hazardous Substances Data Bank: Hansch, Leo et al.: 0.65).[55,62,63] Davis and Papich et al. reported a log P of −1.12[64] while Kasim et al. published a log P of −0.67 and a Clog P of −1.64.[65] The latter values were estimated with the help of ChemDraw Ultra 6.0 and correlated with effective human jejunal permeability data from the literature. The authors of this study reported that the classification of cephalexin as poorly permeable, based on the partition coefficients, is falsely negative, which is in accordance of the carrier-mediated transport mechanism of this polar molecule.

Yamazaki et al. reported values for the distribution coefficient (log D) of the anhydrous substance obtained by calculations with ACD Physichem Batch.[66] The values of −4.94, −2.24 and −10.22 were found at pH 0, 7, and 14, respectively. The log P in this study was also reported to be 0.65.

Stability and Degradation

The stability of cephalexin as the monohydrate depends on the storage conditions (temperature and relative humidity) as outlined above. Degradation of the cephalexin molecule structure is catalyzed by heat, strong acids or bases and UV light at a wavelength of 260 nm.[19] The stability of cephalexin in aqueous solutions is pH-dependent. In the pH range 3–5, solutions of the drug at 25 °C were reported to be stable over 72 h.[19,20] A low degradation rate in the same pH range at an ionic strength of 0.5 was also found at 35 °C.[56] Griffith et al. reported that cephalexin has highest stability in a USP standard buffer at pH 4.5 (close to its isoelectric point) at 25 °C with only slight degradation after 7 days.[32] At pH 6 and 7 at 25 °C, a degradation of 3 and 18% per day, respectively, was reported.[20] The highest extent of degradation has been reported for basic aqueous solutions.[20,56]

The instability of cephalexin in water is due to various chemical reactions. Acidic degradation plays only a minor role in cephalexin degradation, even in strongly acidic solutions.[56] The water-catalyzed degradation of the β-lactam structure is relevant in neutral solutions as well as in the pH range of 3–5. Yamana et al. also discussed an intramolecular reaction involving the side-chain amido group in a neutral environment. An intramolecular nucleophilic attack of the side chain on the β-lactam structure at pH 8 and above and hydroxide-ion-catalyzed degradation above a pH value of 10 was also reported in this study. Typical products of neutral and alkaline degradation are diketopiperazines (Piperazine-2,5-dionene)[20,56] and hydroxy-4-methyl-2(5H)-thiophenone.[20]

HPLC chromatograms that were generated during pilot solubility studies at pH 6.8 in studies performed for this monograph showed peaks with a changed shape compared to chromatograms of standard cephalexin solutions, as well as various small peaks eluting after the main substance peak. The observed patterns were in line with HPLC chromatograms of degraded cephalexin monohydrate reported by Yamana et al.[56] Altered chromatograms were also observed at pH 4.5, but less obvious. Subsequently, degradation studies were performed in pH 4.5 and 6.8 buffers. At pH 4.5, ~4% degradation was observed after 3 4 hours, whereas at pH 6.8 ~7% was degraded after 3 hours and ~10% after 4 hours. Therefore, solubility studies with a shorter duration were conducted at both pH values. Following the FDA guidance regarding degradation issues at intestinal pH conditions,[5] a period which reflects the time span relevant to the time during which the drug could be absorbed from the upper small intestine (for cephalexin estimated at max. 3 hours) was applied to the additional solubility studies.[67]

Because of degradation issues during dissolution tests and HPLC analysis of the samples, the protocol for the dissolution tests was also adapted at pH 4.5 and 6.8 to minimize the time between sampling and analysis (see section on *in vitro* dissolution studies).

Solubility

The different hydrates/solvates (pseudopolymorphic forms) of cephalexin, especially the hydrates, differ in their solubility.[68] Furthermore, the solubility depends on the crystallinity of the cephalexin sample.[46] The European Pharmacopoeia states that cephalexin monohydrate is "sparingly soluble" in water which means a solubility of 1 g in 30–100 mL water at 15–25 °C.[14] The commentary to the Ph. Eur. mentions a solubility of 1 g in 74 mL water at 25 °C.[20] Griffith et al. reported with reference to a personal communication with Pfeiffer, Tucker and Yang that only 1 or 2 mg of cephalexin can be easily dissolved in 1 mL of water at 37 °C, with the solubility increasing at lower or higher pH values (120 mg/mL at pH 2 and 100 mg/mL at pH 8) due to the zwitterionic structure of the molecule.[32] Other literature sources also report pH-dependent solubility values for cephalexin monohydrate. The Ph. Eur. commentary presents values of 120 mg/mL at pH 2.3, 12 mg/mL at pH 5.0 and 100 mg/mL at pH 8.2 without specification of the temperature.[20] Tsuji et al. provided solubility curves over a range of pH 2–8 at an ionic strength of 0.5 at 37 °C and reported the lowest solubility (17.2 mg/mL) at the isoelectric point (in this case reported as pH 4.72).[61] To assess the solubility relevant to the biowaiver guidelines, the solubility profile over the pH range of 1–6.8 at 37 °C has to be established. To verify and extend the solubility values found in the pharmaceutical literature, additional solubility studies were conducted. Table 1 shows the results of those experiments.

Dosage Form Strengths

The 20th edition of the EML published by the WHO lists a solid oral dosage form of 250 mg cephalexin (manufactured as the monohydrate) as a key public access antibiotic.[2] Capsules containing 250, 333, 500, or 750 mg cephalexin and tablets of 250, 500, or 1000 mg cephalexin are available in various international drug markets.[62] In Germany, 2 manufacturers have registered generic tablet formulations of cephalexin.[69] Cephalex-CT is available with 526 or 1052 mg cephalexin monohydrate corresponding to 500 and 1000 mg anhydrous cephalexin, respectively. The same dosage

Table 1
Minimum Solubility of Cephalexin Monohydrate Determined at 37 ± 1 °C Over a pH Range of 1.0–6.8 Over 24 (3) Hours

Medium	Initial pH	Final pH	(Minimum) Solubility 24 h (mg/mL)	(Maximum) D/S Ratio (mL) 24 h D = 1000 mg	(Minimum) Solubility 3 h (mg/mL)	(Maximum) D/S Ratio (mL) 3 h D = 1000 mg
Hydrochloric Acid Buffer[a]	1.0	1.0	>4.15	241 ± 5		
Hydrochloric Acid Buffer[b]	1.2	1.1	4.09 ± 0.33	246 ± 21		
Hydrochloric Acid Buffer[b]	1.7	1.7	4.36 ± 0.3	230 ± 17		
Phosphate Buffer[c]	2.7	2.8	4.65 ± 0.52	217 ± 26		
Phthalate Buffer[c]	3.7	3.7	3.90 ± 0.46[e]	259 ± 29[e]		
Acetate Buffer[d]	4.5	4.7	2.57 ± 0.28	393 ± 46	4.13 ± 0.71	247 ± 47
Phosphate Buffer[b]	6.8	6.6	2.38 ± 0.08	420 ± 15	4.14 ± 0.31	243 ± 18

Experiments were conducted in triplicate (n = 3). Solubility data are expressed as mean ± SD, except where the minimum solubility is reported.
[a] Standard buffer solution (USP) with pH adjusted to initial pH.
[b] Standard buffer solution (USP).
[c] Buffer solution (Ph. Eur.) with pH adjusted to initial pH.
[d] Buffer solution (Ph. Eur.).
[e] A degradation experiment (n = 1) showed a higher solubility and therefore a lower D/S ratio after 3 hours.

strengths are available for Cephalexin-ratiopharm®. All 4 formulations are film-coated tablets. The hydrochloride salt of cephalexin is also used in solid oral dosage forms (doses of 1.16 g cephalexin hydrochloride are equivalent to an amount of 1 g anhydrous cephalexin), while the lysine and sodium salts are found in parental formulations.[18]

Pharmacokinetic Properties

Absorption and Bioavailability

Cephalexin monohydrate is rapidly and almost completely absorbed, since it is a substrate of the intestinal proton-coupled oligopeptide transporter PEPT1, which is located on the enterocytes.[1,51,52,70,72] The α-amino group of cephalexin is sufficiently peptidomimetic to facilitate its interaction with the carrier.[72] Hence, the development of a prodrug, for example the pivaloyl ester, is not required.[73]

The pharmaceutical literature reports an absolute bioavailability for cephalexin of greater than 90% after oral administration.[74] The AUC of cephalexin after a single oral dosage of 1000 mg was determined by Lode et al. in 12 healthy volunteers as 93.0 ± 14.8 h*mg/L for a period of 8 hours (mean ± standard deviation (SD), n = 12).[75] Pfeffer et al. reported AUC values for single doses of 250 and 500 mg cephalexin given orally of 14.0 ± 0.6 h*µg/mL (8 hours, mean ± SE, n = 16) and 29.0 ± 2.5 h*µg/mL (12 hours, mean ± standard error of the means (SE), n = 12), respectively.[76] Other studies published values after multiple doses of 1000 mg cephalexin in capsules and in tablets, that is 3165.9 ± 561.5 min*µg/mL and 3080.7 ± 417.9 min*µg/mL (AUC of the dosing interval of 6 hours, 9 subjects, mean ± SD).[77]

Consistent values for the maximum concentration (c_{max}) of cephalexin in plasma or serum and the corresponding values for the time at which the maximum concentration occurs (t_{max}) have been reported in healthy adult volunteers. After oral single doses of 250 mg cephalexin, maximum plasma levels of 9.93 ± 0.76 µg/mL (t_{max} 0.86 ± 0.05 h; values are mean ± SE, n = 16),[76] 6.8 µg/mL (t_{max} 1 h),[29] 8.4 µg/mL (t_{max} 1 h)[31] and 9 mg/L (t_{max} 1 h)[34,36] have been observed. 500 mg of cephalexin administered in solid oral dosage forms resulted in maximum plasma levels of 20.7 ± 1.5 µg/mL (t_{max} 0.71 ± 0.06 h, mean ± SE, n = 12),[76] 18.8 µg/mL (t_{max} 1 h),[31] 18 mg/L (t_{max} 1 h),[18,27,34,36] 17.6 µg/mL (t_{max} 1 h)[29] and 15.9 µg/mL (t_{max} 1 h) after repeated doses.[27] C_{max} values for orally administered single doses of 1000 mg were reported to be 38.8 ± 8.1 mg/L (t_{max} 55.5 ± 21.8 min; mean ± SD, n = 12),[75] 31.6 µg/mL (t_{max} 1 h),[78] 25.0 µg/mL (t_{max} 1 h)[29] and 32 mg/l (t_{max} 1 h).[1,34,36] Finkelstein et al. reported comparable values for each individual at various time points during multiple doses of 1000 mg cephalexin.[77] Those values verify that the absorption of cephalexin is dose-proportional over the dose range 250–1000 mg, as has been reported in other literature[1,36,76] and which is also the basis for many bioequivalence studies (see also Table 2).

Cephalexin monohydrate shows a food effect. The maximum concentration is diminished and the t_{max} is prolonged if cephalexin is administered with food. This can be interpreted as a negative food effect.[1,26,27,75,78] On the other hand, many sources report that the extent of absorption and the area under the plasma concentration profile are not significantly decreased by administration with food.[1,35,73,76,78] In addition, the package insert does not preclude ingestion with food.[35,36] Therefore, the negative food effect does not seem to be of clinical importance.

In newborns and infants up to 6 months, there is not only a reduced rate of absorption but also of excretion.[24] Reduced absorption of drugs in very young children arises from a slower gastric emptying rate in newborns and infants.[79] Referring back to the negative food effect seen in adults, it can be hypothesized that slower gastric emptying could also explain the altered PK in the fed state in newborns and infants.

Early publications reported amounts of unchanged cephalexin in the urine from 41% to 100% or more within 6 to 8 h after the oral administration of single doses of 250 mg, single and multiple doses of 500, or single and multiple doses of 1000 mg cephalexin in healthy subjects.[26,27,29,31,78] The administration of the drug with food reduced the amount of excreted cephalexin in the different collection periods.[27,75,78] More recent references report values of 80–90% urine recovery after oral administration.[33,34,36,74,75,77] Similarly, 80% of a dose of cephalexin was found unchanged in the urine after a 3-hour infusion (0.5 g/h),[80] while in other studies 96% was found unchanged in the urine within 24 hours after a 20 minute infusion (0.5 g).[81,82] Gower et al. found recovery values after the intravenous and oral administration of 1 g cephalexin to 5 fasting volunteers of 55.5–101.7% (intravenously) and 79.6–111.3% (orally), respectively.[83]

Permeability

A literature search for permeability data of cephalexin monohydrate revealed a value for the human jejunal permeability of 1.56×10^{-4} cm/s which was obtained by a single-pass *in situ* human intestinal perfusion study using propranolol as the reference compound.[65,84] Permeability studies using rat jejunum and Caco-2 cells showed different results. In a single-pass *in situ* rat jejunum

Table 2
Summary of Bioequivalence Studies on Cephalexin Immediate-Release Solid Oral Dosage Forms Available in the Open Pharmaceutical Literature

Reference	Formulations	Subjects	Pre-/Post-Prandial	Study Design	Assessed Pharmacokinetic Parameters	Bioequivalence Criteria	*In Vitro*-Testing	Results[a]
Suleiman et al. (1988)[93]	500 mg: • Keflex tablets (Eli Lilly and Co. Ltd., UK) • Cephadar capsules (Dar Al Dawa Ltd., Jordan) • Lexin capsules (Al-Hikma Pharmaceuticals, Jordan) • Medolexin capsules (Medochemie Ltd., Cyprus) • Ospexin tablets (Biochemie, Austria) • Ultrasporin capsules (ArabPharmaceutical Col Ltd., Jordan)	Six volunteers (healthy, male, adult [26–37], normal [2 smokers]) body weight range 58–95 kg	Fasted (10 h before water, fruit juice, milk freely available)	Randomized, crossover, 6 trials, 1 week wash-out	• Cumulative amount excreted after 7 h (mg) • Urinary peak height (mg/h) • Time to reach peak (h) • Elimination and absorption rate (h^{-1})	Two-way ANOVA, Duncan's new multiple range method, $p < 0.05$	No	Bioequivalent (BA difference < 20% from reference product Keflex tablets)
Blume et al. (1995)[94]	1000 mg: • Cephalexin ratiopharm tablets (ratiopharm GmbH) • Oracef tablets (Dr. Karl Thomae GmbH)	10 volunteers (3 male, adult [18–44] body weight range 55–80 kg)	Fasted	Crossover, single dose, two-period, wash-out 1 week	• C_{max} (mg/l); • AUC_t (mg × h/l); • AUC_∞ (mg × h/l) • t_{max} (h)	Similar curves (mean values), ANOVA, $ANOVA_{log}$, Wilcoxon signed rank test, 90% CI (AUC 80–125%, c_{max} 70–143%), no therapeutic differences in t_{max}, AUC_{res} <20%	No	Bioequivalent
Fachinformation Cephalexin-ratiopharm 500 mg/ 1000 mg (study of 1998)[95,b]	• Cephalexin ratiopharm 1000 mg film-coated tablets • Reference product (not specified)	20 volunteers	Not specified	Not specified	• C_{max} (μg/mL) • t_{max} (h) • AUC (h × μg/mL)	Not specified	No	Bioequivalent in AUC, c_{max} and t_{max} dose-linearity for 500 mg according to CPMP guideline
MHRA Public Assessment Report (2009)[95,c]	500 mg: • Cefalexin capsules (Orchid Healthcare LTD.) • Keflex capsules (Eli Lilly Ltd.)	24 volunteers (healthy, male, adult)	Fasted	Randomized, crossover, single dose, two-way, two-period, 7 days wash-out	• C_{max} (μg/mL) • AUC_t (μg × h/mL) • AUC_∞ (μg × h/mL) • t_{max} (h)	ANOVA, 90% CI (0.8 −1.25 acceptance interval for AUC and c_{max})	Yes, not specified	Bioequivalent Linear kinetics for 250 mg
Mohamed et al. (2011)[96]	500 mg: • Amilexin capsules (Amipharma Lab. Ltd., Sudan) • Sigmacef capsules (Sigma Tau Sudan Ltd.) • Keflex capsules (Eli Lilly Ltd.)	24 volunteers (healthy, male, adults [20 −38], Sudanese) body weight range 44–70 kg	Fasted	Open, randomized, crossover, single dose, 1 week wash-out	Ratios of • c_{max} (μg/mL) • AUC_8 (μg × h/mL) • AUC_∞ (μg × h/mL)	One-way ANOVA, 2 one-sided t-tests, 90% CI (FDA acceptance interval 80.00–120.00%), $p < 0.05$	No	Bioequivalent
MHRA UK Public Assessment Report (2011)[97,d]	500 mg: • Cefalexin capsules (Lupin Ltd.) • Keflex capsules → Cefalexin capsules (Eli Lilly Ltd. → Flynn Pharma Ltd.)	27 volunteers (healthy, male, adult)	Fasted	Open-label, randomized, crossover, single dose, two-treatment, two-way, two-period, 4 days wash-out	Log-transformed • c_{max} (μg/mL) • AUC_t (μg × h/mL) • AUC_∞ (μg × h/mL)	90% CI (acceptance interval 80.00 −125.00%)	Yes, not specified	Bioequivalent extrapolated for 250 mg

(*continued on next page*)

Table 2 (*continued*)

Reference	Formulations	Subjects	Pre-/Post-Prandial	Study Design	Assessed Pharmacokinetic Parameters	Bioequivalence Criteria	*In Vitro*-Testing	Results[a]
MEB Public Assessment Report (2012)[98,e]	500 mg: • Cefalexine EQL Pharma, fil-coated tablets (EQL Pharma AB) • Keflex tablets (Eli Lilly Ltd.)	24 volunteers (healthy, male)	Fasted	Randomized, crossover, single dose, two-treatment, two-sequence, two-period, 9 days wash-out	In-transformed ratios: • AUC_{0-t} (μg × h/mL) • $AUC_{0-\infty}$ (μg × h/mL) • c_{max} (μg/mL)	90% CI (acceptance interval 0.80–1.25)	Yes, not specified	Bioequivalent dose-proportionality for 250 mg, 750 mg, and 1000 mg (biowaiver)
Sethi et al. (2013)[99]	500 mg: • Zeporin capsules (Z-Jans Pharmaceuticals Ltd.) • Ceporex capsules (GSK)	Healthy, male volunteers from local population (Pakistan)	Fasted	Randomized, crossover, two-way	Log-transformed: • c_{max} (μg/mL) • AUC_{0-t} (μg × h/mL) • $AUC_{0-\infty}$ (μg × h/mL) • t_{max} (h)	ANOVA, 90% CI (acceptance interval 0.80–1.25), Wilcoxon signed rank test (t_{max}), $p < 0.05$	Yes USP Apparatus I, 900 mL distilled water, 37 ± 0.5 °C, 100 rpm, 5 mL samples at 10, 20, 30, 40, 50, and 60 min, UV spectroscopic analysis <85% in 15 min, >85% in 30 min	Bioequivalent
MHRA Public Assessment Report (2013)[100,f]	500 mg: • Cefalexin capsules (Alkem Pharma GmbH) • Keflex capsules (Eli Lilly Ltd. → Flynn Pharmaceuticals Ltd.)	Healthy, adult volunteers	Fasted	Open-label, laboratory-blind, pivotal, balanced, randomized, crossover, single dose, two-treatment, two-way, two-sequence, two-period, 7 days wash-out	Log-transformed (geometric least square means) ratios: • c_{max} (ng/mL) • AUC_t (ng × h/mL) • AUC_∞ (ng × h/mL)	90% CI (acceptance criteria according to "Guideline on the Investigation of Bioequivalence")	Yes, not specified	Bioequivalent dose-proportionality for 250 mg
Liew et al. (2014)[101]	500 mg: • MPI Cephalexin tablets (Malaysian Pharmaceutical Industries Sdn Bhd.) • MPI Cephalexin capsules (Malaysian Pharmaceutical Industries Sdn Bhd.) • Ospexin tablet (Sandoz)	24 volunteers (healthy, male, audlt [20–41], Malays) body weight range: 56.4–90.0 kg	Fasted	Open label, randomized, crossover, single dose, three-treatment, three-period, three-sequence, 1 week wash-out	In-transformed ratios: • AUC_{0-t} (μg × h/mL) • $AUC_{0-\infty}$ (μg × h/mL) • c_{max} (μg/mL)	ANOVA, 90% CI interval, $p < 0.05$	No	Bioequivalent
Alhamd (2014)[102]	500 mg: • Cephalexin capsules (SDI, Iraq) • Cephalexin capsules (Anjanta Pharm. Ltd.)	20 volunteers (healthy, male, Iraqi) age range 25–45 body weight range 70–95 kg	Fasted	Single dose	Ratios of • c_{max} (ppm) • t_{max} (h) • AUC	90% CI (FDA acceptance interval 80–120%)	No	Bioequivalent
dos Reis Serra et al. (2015)[103]	500 mg tablets available in the Brazilian market	24 volunteers (healthy, male and female) age rage 21–35 body weight range 49–84 kg	Fasted	Open, randomized, crossover, single dose, two-period, Latin square design, 2 weeks wash-out	• Cumulative amount of excreted cephalexin (Du_C) • Total cumulative amount of cephalexin excreted (Du_{∞}) • Maximum excretion rate of cephalexin $[(dDu/dt)_{max}]$	ANOVA, 90% CI (ratio of Du_C, Du^{∞}, $(dDu/dt)_{max}$ [acceptance interval 80–125%])	Yes 6 tablets, apparatus I (basket, 40 mesh), 900 mL distilled water, 37.0 ± 0.5 °C, 100 rpm, sampling at 5, 7, 10, 15, 20, 30, 40, 45, 50 and 60 min, UV spectrophotometric	Bioequivalent

					• Middle-time of maximum excretion rate of cephalexin (tm_{max}) • Excretion constant rate of cephalexin (k) • Cephalexin elimination half-life ($t_{½}$)[b]		analysis <80% in 30 mins	
Hammami et al. (2017)[104]	500 mg: One reference and 3 randomly selected generic immediate-release, non-combinational, oral products of the Saudi market	36 volunteers (healthy, male, adults [18–60]) Body mass index ≤35 kg/m^2	Fasted	Sequence-randomized, crossover, four-product, four-sequence, four-period	• C_{max} • AUC_T • AUC_I	ANOVA, 90% CI interval (geometric mean ratio: MSR with/ without Bonferroni adjustment [acceptance criteria 80.00–125.00%])	No	Reference-generic and generic-generic bioequivalent

[a] Bioequivalence was assessed by the authors/health authorities of the references. The criteria for the assessment may differ from the current criteria for bioequivalence published by the various health authorities.
[b] Composition of Cephalexin ratiopharm 1000 mg: Macrogol 6000, magnesium stearate, carboxymethyl starch sodium (type A), povidone, lactose monohydrate, saccharin sodium, peppermint oil, titanium dioxide, talc, hypromellose.
[c] Composition of Cefalexin 250 mg/500 mg capsules (Orchid Europe Ltd.): microcrystalline cellulose, croscarmellose sodium, magnesium stearate (capsule content); Brilliant Blue FCF (E110), titanium dioxide, gelatin, sodium lauril sulfate (capsule shell); shellac, dehydrated alcohol, isopropyl alcohol, propylene glycol (black printing).
[d] Composition of Cefalexin 250 mg/500 mg capsules (Lupin Ltd.): microcrystalline cellulose, magnesium stearate, water (capsule core); gelatin, sodium lauryl sulfate, sunset yellow FCF (E110), quinoline yellow (E104), titanium dioxide E171), patent blue V (E131) (capsule shell); shellac, propylene glycol, potassium hydroxide, black iron oxide (E172) (black ink).
[e] Composition of Cefalexine EQL Pharma 250 mg/500 mg/750 mg/1000 mg, film-coated tablets (EQL Pharma AB): sodium starch glycolate (Type A), microcrystalline cellulose (E460), magnesium stearate (tablet core); hypromellose (E464), titanium dioxide (E171), yellow iron oxide (E172), red iron oxide (E172), glycerol (E422), talc (film-coating).
[f] Composition of Cefalexin capsules (Alkem Pharma GmbH): Lactose anhydrous (SuperTab 21AN), colloidal silicon dioxide, magnesium stearate, gelatin, sodium laurylsulfate, water, FD.&C Blue 1 (E 133), FD & C Red 40 (E 129), D & C Yelloq 10 (E 104), titanium dioxide (E 171); shellac, dehydrated alcohol, isopropyl alcohol, butyl alcohol, propylene glycol, strong ammonia solution, black iron oxide (E 172), potassium hydroxide, purified water (black ink).

perfusion study, the mean P_{eff} of cephalexin was calculated to be $3.89 \pm 1.63 \times 10^{-5}$ cm/s (mean value ± SD, n = 15).[70] Raeissi et al. determined the apical-to-basolateral permeability coefficient of cephalexin using Caco-2 cells cultured on polycarbonate transwell inserts in the presence and absence of a pH gradient.[72] They reported permeability coefficients of $7.58 \pm 0.85 \times 10^{-7}$ cm/s at pH 6.0 and $2.73 \pm 0.39 \times 10^{-7}$ cm/s at pH 7.4 (The publication did not indicate the size of n and whether SD or SE were reported). Another study compared the permeability data of cephalexin obtained by Caco-2 cell experiments with monolayers that were cultured at different cell differentiation times performed at pH values of 6.5 and 7.4 and with data obtained by bio-mimetic artificial membrane permeability assay (BAMPA) experiments at pH 5.5, 6.5 and 7.4.[71] Permeability coefficients of $9.21 \pm 4.42 \times 10^{-6}$ cm/s (Caco-2 cells, 3 days, pH 6.5; mean ± SE, n = 2) and $42.05 \pm 8.72 \times 10^{-6}$ cm/s (Caco-2 cells, 21 days, pH 6.5; mean ± SE, n = 3) and of $1.10 \pm 0.13 \times 10^{-6}$ cm/s (BAMPA, pH 5.5; mean ± SE, n = 3) and $0.97 \pm 0.12 \times 10^{-6}$ cm/s (BAMPA, pH 6.5; mean ± SE, n = 3) were reported, respectively. To interpret these results properly, the under-expression of active transporters in Caco-2 cells must be taken into account, while artificial membrane studies cannot detect active transport uptake mechanisms.

Distribution

Cephalexin is widely distributed in most tissues of the body, especially in the plasma and extracellular water, liver and kidneys.[24] It crosses the placenta and can be found in breast milk in low concentrations,[74] but barely crosses the blood brain barrier.[85] Its serum protein binding is low, with reported values lying in the range 6–20% depending on the plasma concentration.[32,73,74] Based on a re-evaluation of existing literature data, Nightingale et al. calculated a volume of distribution (V_d) at steady state of 18.4 L (composed of 10.8 L for the central compartment and 7.6 L for the tissue compartment) using a two-compartment model analysis.[24,86] In a study in 10 healthy volunteers, the same research group published a value for the volume of distribution at steady-state of 16.4 L after intravenous administration of cephalexin. A value of 13.7 L was reported for oral administration.[86] These values are in the same range as the value of 15 L (±2.3 standard deviation) reported by Kirby et al.[80,81] and by Bergan.[74] Volume of distribution values based on analysis of the β-phase of the two-compartment model and extrapolated V_d values are higher than the values reported above (19.6–23.3 L and 24.7–31.7 L, respectively[24,86]). Other research groups have reported similar values.[19,75]

Metabolism and Elimination

Cephalexin is almost completely excreted in the urine[32,81] and metabolic pathways have not been reported in the pharmaceutical literature. It is excreted mainly via glomerular filtration but tubular secretion also plays a role since the cephalexin clearance is higher than the creatinine clearance.[19,24,32,34-36,78,80,81] The clearance that is achieved by this major pathway of elimination was calculated by DeMaine and Kirby to be 210 ± 32 mL/min/1.73 m^2 which is approximately 72% of the serum clearance of 291 ± 22 mL/min/1.73 m^2 (mean values ± standard deviation).[80,81] These values were obtained after an intravenous infusion (0.5 g/h). Comparable values were reported by Kirby and Regamey, that is 252 ± 5 mL/min/1.73 m^2 (renal clearance) and 248 ± 11 mL/min/17.73 m^2 (serum clearance), respectively, after a 20-min infusion of 0.5 g cephalexin in 4 volunteers (values ± SE).[82] A further value for the renal clearance of 260 mL/min after intravenous injection of 1000 mg cephalexin sodium to 6 volunteers is in the same range.[83] Individual values for the renal and total clearance after oral administration of cephalexin are also found in the pharmaceutical literature: 308.5 ± 94.8 mL/min and

325.7 ± 56.0 mL/min (renal clearance and total body clearance, 1000 mg cephalexin in capsules every 6 h for 5 doses, 9 volunteers, mean ± SD),[77] 321.3 ± 103.3 mL/min and 329.7 ± 46.5 mL/min (renal clearance and total body clearance, 1000 mg cephalexin from 1 tablet every 6 h for 5 doses, 9 volunteers, mean ± SD),[77] and 376 mL/min (plasma clearance, 1000 mg administered orally, 1 volunteer).[78] From these values and by comparison of dose with urinary recovery, another pathway of excretion must be additionally considered. Although cephalexin is also excreted with the bile, this accounts for only 0.5–1% of the dose.[73,74] Animal studies in rats detected a greater amount of cephalexin (15%) in the feces that had apparently been excreted by the bile,[87] suggesting the possibility of direct excretion via the intestines.

Cephalexin has a short elimination half-life. Therefore, flip-flop kinetics may interfere with determinations of elimination half-life after oral administration. Indeed, calculations based on data after oral administration to healthy subjects show a range of 0.51–1.2 hours (= 30.6–72 minutes),[35,73-76,78,88] whereas half-life values determined after infusion or injection of cephalexin tend to be slightly shorter, 0.6–0.9 hours.[80-83]

The risk of accumulation of cephalexin in healthy patients after repeated doses of cephalexin is low due to the short half-life of the drug.[77] However, in persons with renal impairment or renal failure, this risk is increased because of the longer elimination half-life.[30,74,85,88] Reduced urine levels and enhanced peak serum concentrations can also be caused by an administration of cephalexin with or after probenecid, which inhibits the tubular secretion of the API.[1,26,27,34-36,78] Thus, in cases of renal insufficiency, the dosing scheme must be adjusted according to the extent of renal impairment. Furthermore, the use of probenecid should be avoided during a treatment with cephalexin.

Dosage Form Performance

Bioequivalence Studies

Literature research revealed numerous bioequivalence studies of cephalexin products. Studies including non-solid, oral cephalexin products or studies in which the description of the study protocol was not publicly available[89-92] were not considered for the assessment of cephalexin as a candidate for the BCS-based biowaiver. Table 2 lists a summary of relevant studies on immediate-release, solid oral cephalexin products. Most of the studies reviewed reported the pharmacokinetic parameters AUC, c_{max} and t_{max} calculated from plasma levels of cephalexin over time; other studies reported pharmacokinetic data obtained from the amount of cephalexin excreted in the urine. Bioequivalence was assessed on basis of the statistical evaluation of the pharmacokinetic data. Most of the studies demonstrated that the 90% confidence intervals of the AUC and c_{max} of the test products lay within the 80–120% or 80–125% acceptance interval around those of the comparator. All studies reported bioequivalence for the tested products compared to the chosen reference products.

Excipients

Numerous cephalexin products, including many generic products, are available on the market. The WHO Prequalification List does not list finished pharmaceutical products of cephalexin monohydrate.[105] The excipients which are used in representative cephalexin monohydrate immediate-release solid oral products with a marketing authorization (MA) in Belgium, Canada, Germany, Denmark, Spain, Finland, France, Croatia, Ireland, Iceland, Italy, The Netherlands, Norway, Poland, Romania, Slovakia, United Kingdom and the United States are summarized in Table 3.

Of the excipients specified in this table, polysorbate 80 and sodium lauryl sulfate are suspected to affect the permeability of drugs; as surfactants they could enhance the penetration of drugs though membranes. Since cephalexin monohydrate is actively absorbed, with near 100% bioavailabilty and is thus considered highly permeable, any increase in permeability due to these 2 excipients is not expected to make a discernible difference to the absorption of cephalexin. Addition of surfactants could also increase the wettability and dissolution rate of poorly wettable drugs, but such effects would be detected during dissolution testing when applying the BCS-based biowaiver methods. When magnesium stearate, a hydrophobic glidant, is used in larger quantities, this can lead to poorer dissolution and hence lower absorption. Again, this effect would be detected in dissolution experiments. On the other hand, macrogols could negatively affect the absorption of drugs due to reduced transit time, which results from increased intestinal motility because of the osmotic properties of this group of excipients. This effect would not be detected with dissolution experiments. However, these effects are only observed at high concentrations of low molecular weight macrogols, which are unlikely to be present in solid oral formulations in large amounts. Further, since all these products have marketing authorizations in ICH and associated countries, it may be assumed that, despite the use of the above-mentioned excipients, the products are all bioequivalent with the respective reference formulation.

Dissolution

The dissolution performance of cephalexin monohydrate was evaluated with the pure API, and with 2 products with marketing authorization in Germany to detect potential influences of excipients or the manufacturing process on the dissolution behavior. Cephalex-CT and Cephalexin-ratiopharm®, both film-coated tablets, each contain 1052 mg cephalexin monohydrate per tablet (equivalent to 1000 mg cephalexin base) and are therefore the highest dosage strengths available on the market.[35,36] The dissolution tests of drug substance and products were all performed at pH values of 1.2, 4.5 and 6.8 in triplicate. Due to degradation issues, dissolution samples obtained at pH values of 4.5 and 6.8 were analyzed immediately after withdrawal. The results of these studies are shown in Figure 2. At all 3 pH values, 85% or more of the pure API was dissolved within 15 minutes. For the tablets, 85% or more was dissolved within 15 minutes at pH 1.2 and 6.8, while disintegration problems resulted in failure of both the CT and Ratiopharm tablets to release 85% of the labelled amount of cephalexin within 15 or 30 minutes at pH 4.5.

Discussion

Solubility

The cited literature solubility data report values in excess of 4 mg/mL at different pH values in a range of 1–6.8 at 37 °C, which indicates that the highest strength of cephalexin monohydrate (which is also the highest single therapeutic dose [1000 mg]) would be soluble in 250 mL medium over this pH range. In order to arrive at a complete guideline-conform solubility profile followed by the calculation of the dose/solubility ratios, the 24-h solubility at pH 1, 1.2, 1.7 (= pK_a-1), 2.7 (= pK_a), 3.7 (= pK_a+1), 4.5 (in the case of cephalexin also the IEP), and 6.8 at 37 °C was established.

The values for the D/S ratio are below 250 mL at pH values below 3.7. The 24-h solubility values at pH 3.7, 4.5 and 6.8 exceeded the cut-off value of 250 mL. In terms of the studies at pH 4.5 and 6.8, degradation reactions were observed in both the samples and the chromatograms of the quantitative analysis. The color of the initially colorless saturated solutions turned yellow and a sulfurous odor was noticed. The chromatograms showed additional peaks

Table 3
Excipients[a] Present in Cephalexin IR Solid Oral Drug Products[b] With a Marketing Authorization (MA) in Belgium (BE), Canada (CA), Germany (DE), Denmark (DK), Spain (ES), Finland (FI), France (FR), Croatia (HR), Ireland (IE), Iceland (IS), Italy (IT), The Netherlands (NL), Norway (NO), Poland (PL), Romania (RO), Slovakia (SK), United Kingdom (UK) and the United States (US)[c], and the Minimal and Maximal Amount of That Excipient Present Pro Dosage Unit in Solid Oral Drug Products With a MA in the US[d]

Excipient	Drug Products Containing That Excipient With a MA Granted by the Named Country	Range Present in Solid Oral Dosage Forms With a MA in the US (mg)
Carmellose Sodium	ES([f]) IE([g-i]) UK([j]) US([k,l])	3.2–160
Cellulose, Microcrystalline	CA([m-p]) HR([q,r]) IT([s]) RO([t-v]) UK([w,x]) US([k,l,y-ff])	4.6–1553[e]
Croscarmellose Sodium	US([z-bb,ee])	2.5–180
Dimeticone	ES([f]) IE([g-i]) UK([j]) US([k,l])	2.2–3.7
Glycerol	CA([gg,hh]) UK([ii])	0.6–249
Hydroxypropylcellulose	CA([m,o])	0.2–132
Hypromellose	CA([m-p,gg,hh]) IT([s]) UK([ii]) US([y])	0.2–1943[e]
Lactose	DE([jj,kk]) FI([ll,mm]) RO([nn]) SK([oo]) UK([pp-ss]) US([tt-ddd])	23–2217[e]
Macrogols	CA([o-nn]) DE([jj,kk]) FI([ll,mm]) RO([nn]) SK([oo]) UK([rr,ss]) US([y])	0.13–1057[e]
Magnesium Stearate	BE([eee]) CA([o-nn,gg,hh,fff,ggg]) DE([jj,kk]) DK([hhh]) ES([iii]) FI([ll,mm]) FR([jjj]) HR([q,r,kkk]) IE([g-i,lll-nnn]) IS([ooo]) IT([s,ppp]) NL([qqq]) NO([rrr]) PL([sss]) RO([t-v,nn,ttt]) SK([oo]) UK([s,w,x,ll,pp-ss]) US([k,l,y-ff,tt-ddd,uuu-www])	0.15–79
Methylcellulose	CA([gg,hh])	2.8–184
Polysorbate 80	US([y])	0.1–418[e]
Povidone	BE([eee]) CA([hh]) DE([jj,kk]) DK([hhh]) FI([ll,mm]) FR([jjj]) IE([lll-nnn]) IS([ooo]) IT([ppp]) NL([qqq]) NO([rrr]) RO([nn]) SK([oo]) UK([rr,ss])	1–240
Silica	CA([m,o,p,fff,ggg]) HR([r]) RO([ttt]) US([ff,vv-xx,aaa,bbb,ddd,uuu,www])	0.1–138
Sodium Lauril Sulfate	CA([fff,ggg]) HR([kkk]) US([z-bb,vv-xx])	0.26–96
Sodium Starch Glycolate	BE([eee]) CA([n,gg,hh]) DE([jj,kk]) DK([hhh]) FI([ll,mm]) FR([jjj]) HR([r]) IE([lll-nnn]) IS([ooo]) IT([ppp]) NL([qqq]) NO([rrr]) RO([nn]) SK([oo]) UK([ll,rr,ss]) US([y,aaa,bbb,uuu,www])	2–876[e]
Starch	CA([gg]) UK([ii])	0.44–616[e]
Starch, Modified	CA([fff,ggg]) UK([ii])	23–50
Starch, Pregelatinised	CA([p,gg]) UK([ii])	9.4–453
Stearic Acid	CA([m,o,p,gg]) UK([ii])	0.9–72[e]
Talc	CA([gg,hh]) HR([r]) PL([sss]) RO([u,ttt]) UK([ii,rr,ss]) US([u,u,yy-ccc])	0.1–321[e]

[a] Colorants, flavors, water, and ingredients present in the coating and the printing ink are not included. Substances are excluded if it can be assumed that the constituents are only present in the coating/polish.
[b] Excluded are: oral suspension and powder for oral suspension.
[c] Sources of data: BE, www.bcfi.be/ (accessed 25-02-2019); CA, https://www.canada.ca/en.html (accessed 25-02-2019); DE, www.rote-liste.de; (accessed 25-02-2019); DK, https://laegemiddelstyrelsen.dk/ (accessed 26-02-2019); ES, www.aemps.es (accessed 26-02-2019); FI, www.fimea.fi (accessed 26-02-2019); FR, www.vidal.fr/; (accessed 26-02-2019); HR, http://www.almp.hu/ (accessed 26-02-2019); IE, https://www.hpra.ie/ (accessed 26-02-2019); IS, https://www.lyfjastofnun.is/ (accessed 26-02-2019); IT, https://www.torrinomedica.it/farmaci-trovati (accessed 26-02-2019); NL, www.cbg-meb.nl. (accessed 26-02-2019); NO, www.legemiddelverket.no/ (accessed 26-02-2019); PL, http://urpl.gov.pl/en (accessed 26-02-2019); RO, www.anm.ro/ (accessed 26-02-2019); SK, www.sukl.sk (accessed 26-02-2019); UK, www.medicines.org.uk/emc/ (accessed 26-02-2019); US, www.dailymed.nlm.nih.gov. (accessed 26-02-2019).
[d] US: FDA's Inactive Ingredient Database, http://www.fda.gov/Drugs/InformationOnDrugs/ucm113978.htm (version date december 2018)
[e] The upper range value reported is unusually high for solid oral dosage forms and the authors doubt its correctness.
[f] KEFLORIDINA FORTE 500 mg, cápsulas duras.
[g] Keflex 250 mg Hard Capsules [Imbat Ltd.].
[h] Keflex 250 mg Hard Capsules [Flynn Pharma Limited]
[i] Keflex 250 mg Hard capsules [LTT Pharma Limited]
[j] Keflex Capsules 250/–500 mg [Flynn Pharma Limited]
[k] Cephalexin Capsules, USP 750 mg [Fera Pharmaceuticals, LLC]
[l] KEFLEX® Cephalexin Capsules, USP 250/–500/–750 mg [Pragma Pharmaceuticals, LLC]
[m] [Pr]APO-CEPHALEX (Cephalexin Tablets USP) 250/–500 mg
[n] [Pr]AURO-CEPHALEXIN (Cefalexin Tablets, BP) 250/–500 mg
[o] [Pr]CEPHALEXIN –500 (Cephalexin Tablets USP) 500 mg
[p] [Pr]LUPIN-CEPHALEXIN 250/–500 mg
[q] CEFALEKSIN Belupo 500 mg kapsule
[r] Cefalin 1 g filmom obložene tablete
[s] CEPOREX 1 g Compresse rivestite
[t] CEFALEXIN SANDOZ 250/–500 mg capsule
[u] Cefalexină Arena 250/–500 mg capsule
[v] Ospexin 250/–500 mg capsule
[w] Cefalexin 250/–500 mg Capsules [Lupin Healthcare (UK) Limited]
[x] Tenkorex Capsules 500 mg/Cefalexin 500 mg Capsules [Sandoz GmbH]
[y] Cephalexin Tablets USP 250/–500 mg [Teva Pharmaceuticals USA, Inc.]
[z] Cephalexin Capsule, USP 500 mg [AMELLA PHARMA, LLC]
[aa] Cephalexin Capsules, USP 250/–500 mg [Aurobindo Pharma Limited]
[bb] Cephalexin Capsules, USP 500 mg [Gen-Source Rx]
[cc] Cephalexin Capsules USP 250/–500 mg [LUPIN LIMITED]
[dd] Cephalexin Capsules USP 250/–500 mg [Lupin Pharmaceuticals, Inc.]
[ee] Cephalexin Capsules USP 250/-500 mg [OrchidPharma Inc]
[ff] KEFLEX- cephalexin capsule 250/–333/–500/–750 mg [ADVANCIS PHARMACEUTICAL CORPORATION]
[gg] [Pr]KEFLEX® (Cephalexin Tablets) 250 mg
[hh] [Pr]KEFLEX® (Cephalexin Tablets) 500 mg
[ii] Keflex Tablets 250/-500 mg [Flynn Pharma Limited]
[jj] Cephalex-CT 500/–1000 mg Filmtabletten
[kk] Cephalexin-ratiopharm® 500/–1000 mg Filmtabletten
[ll] Kefalex 500/–750 mg tabletti, kalvopäällysteinen
[mm] Kefexin 250/–500/–750 mg tabletti, kalvopäällysteinen
[nn] Ospexin 500/–1000 mg comprimate filmate

[oo] Ospexin 500/−1000 mg filmom obalené tablety
[pp] Cefalexin 250/−500 mg Capsules [Milpharm Limited]
[qq] Cefalexin 250/−500 mg Capsules, HARD [Athlone Pharmaceuticals Limited]
[rr] Cefalexin tablets BP 250 mg/Ospexin tablets 250 mg/Tenkorex tablets 250 mg/ Kiflone tablets 250 mg [Sandoz GmbH]
[ss] Ospexin tablets 500 mg/Tenkorex tablets 500 mg/Cefalexin tablets 500 mg/ Kiflone tablets 500 mg [Sandoz GmbH]
[tt] CHEPHALEXIN Capsules, USP 250/−500 mg [Ranbaxy Pharmaceuticals Inc.]
[uu] CEPHALEXIN capsules, USP 250/−500 mg [American Antibiotics, Inc]
[vv] CEPHALEXIN capsules, USP 250/−500 mg [American Health Packaging]
[ww] CEPHALEXIN capsules, USP 250/−333/−500/−750 mg [Ascend Laboratories, LLC]
[xx] CEPHALEXIN capsules, USP 250/−500 mg [AvPAK]
[yy] CEPHALEXIN capsules, USP 250/−500 mg [Belcher Pharmaceuticals, LLC]
[zz] CEPHALEXIN capsules USP, 250/−500 mg [Bi-Coastal Pharma International LLC]
[aaa] CEPHALEXIN capsules USP, 250/−500 mg [Carlsbad Technology, Inc.]
[bbb] Cephalexin Capsules, USP 250/−500 mg [Pharma-C, Inc.]
[ccc] CEPHALEXIN capsules, USP 250/−500 mg [Virtus Pharmaceuticals]
[ddd] Daxbia cephalexin capsules, USP 333 mg [Crown Laboratories, Inc.]
[eee] Keforal 500 mg filmomhulde tabletten
[fff] [pr]TEVA-CEPHALEXIN 250/−500 mg Cephalexin Capsules USP
[ggg] [pr]TEVA-CEPHALEXIN 250/−500 mg Cephalexin Tablets USP
[hhh] Keflex (Cefalexin 500 mg) tabletter
[iii] Cefalexina Normon 500 mg cápsulas duras
[jjj] KEFORAL 500 mg/1 g cp pellic
[kkk] Cefalexin Alkaloid 500 mg tvrde kapsule
[lll] Keflex 500 mg Film-Coated Tablets [Lexon (UK) Ltd]
[mmm] Keflex 500 mg Film-Coated Tablets [Imbat Ltd.]
[nnn] Keflex 500 mg Film-Coated Tablets [Flynn Pharma Limited]
[ooo] Keflex
[ppp] Keforal 500 mg/1 g compresse rivestite con film
[qqq] Keforal 500 mg filmomhulde tabletten
[rrr] Keflex 500 mg tabletter
[sss] CEFALEKSYNA TZF, 500 mg, kapsułki twarde
[ttt] Cefalexină ATB 250/−500 mg, capsule
[uuu] Cephalexin Capsules USP 250/−500 mg [Teva Pharmaceuticals USA, Inc.]
[vvv] Cephalexin Capsules, USP 250/−500 mg [Sun Pharmaceutical Industries Limited]
[www] CEPHALEXIN capsules, USP 250/−500 mg [West-Ward Pharmaceuticals Corp].

that were assigned to degradation products of cephalexin monohydrate. Based on the suggestions of Plöger and Hofsäss et al.[67] and as a consequence of those observations, degradation studies and subsequently 3-h solubility studies were performed. The D/S ratios calculated from the resulting solubility values at pH 4.5 and 6.8 were <250 mL. The solubility value at pH 3.7 can be considered borderline, and in a separate degradation study performed for 4 hours at pH 3.7 a concentration of more than 4 mg/mL (parent drug) was recovered unchanged, indicating a D/S ratio of <250 mL (data not shown). The latter result is in accordance with the literature data. A possible explanation for the borderline solubility might be an undetected incompatibility with the phthalate buffer used at this pH. For further studies, evaluation of the solubility in alternative buffers or in unbuffered solutions with pH correction during the solubility determination should be considered.

Based on the overall literature and experimental data, cephalexin monohydrate can be classified as "highly soluble" according to the BCS-based biowaiver guidelines.

Permeability

According to the latest biowaiver guidelines of the FDA, WHO, and EMA, high permeability can indirectly be assumed when the extent of absorption in humans is 85% or more of an administered dose. For cephalexin, the extent of absorption is over 90% in humans and the pharmacokinetics are linear with dose, both of which are indicative of high permeability. Furthermore, the reported permeability coefficient of 1.56×10^{-4} cm/s from the human intestinal perfusion study confirms the high permeability of cephalexin since drugs with permeability coefficients greater than 1×10^{-6} cm/s can be assumed to be completely absorbed in humans.[106] All reported data in humans are thus consistent with classification of cephalexin as high permeability drug.

Some permeability studies in rats and Caco-2 cells reported lower values as compared to the human jejunal permeability. This may be due to its pH-dependent, carrier-mediated mechanism of intestinal absorption.[70,72] The carrier for cephalexin is the proton-coupled oligopeptide transporter PEPT1 which is found on the apical membrane of enterocytes and is involved in the intestinal absorption of many peptide-like drugs such as β-lactam antibiotics.[51,70] The study of Sun et al. suggested that the low PEPT1 expression in Caco-2 cells as compared to the human duodenum is the reason for the low permeability of carrier-mediated drugs such as cephalexin in Caco-2 cells.[84] Similarly, in the rat jejunum study by Chu et al., it was demonstrated that the variability of cephalexin permeability was due to the variation of PEPT1 expression in rat jejunum.[70] By contrast, in humans the PEPT1 transporter is regarded as a high capacity transporter, explaining why the pharmacokinetics are linear over the usual dose range. The uptake of cephalexin by an active transport mechanism explains why it proved to be an outlier in the correlation of Kasim et al. of human intestinal permeability with Log P and CLog P since these parameters are only able to estimate passive, transcellular absorption.[65] Although cephalexin undergoes degradation in certain buffers as a function of pH (see section Stability and Degradation), degradation is expected to be <10% over usual passage times in the upper gastrointestinal tract.[67] Furthermore, degradation does not seem to pose a limitation to uptake, as underscored by the high bioavailability of cephalexin after oral dosing.

Based on the available human jejunal permeability and its complete absorption in humans, cephalexin can be classified as "highly permeable."

BCS Class

Cephalexin monohydrate can be categorized as "highly soluble" based on the solubility data. The permeability data allow a classification as "highly permeable" drug in accordance with all biowaiver guidelines of the health authorities FDA, WHO and EMA. Since the criteria for solubility and permeability classification in the drafted ICH harmonized guideline for BCS-based biowaivers[107] do not vary

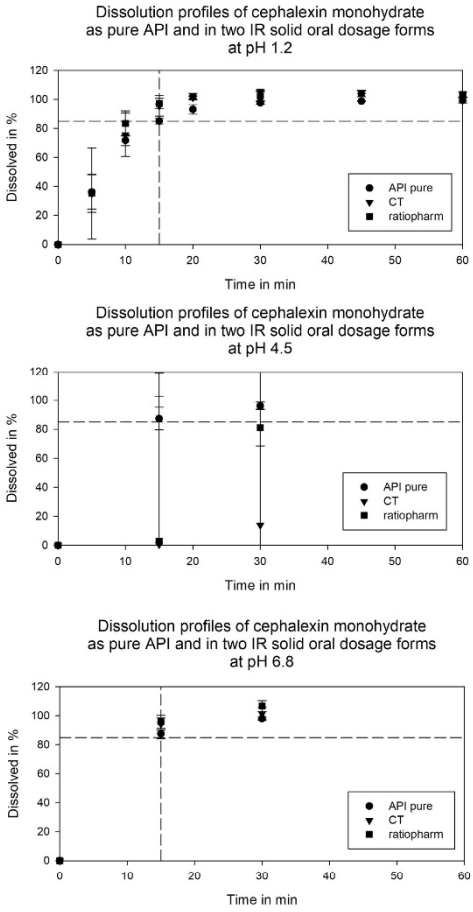

Figure 2. Dissolution profiles of cephalexin monohydrate and 2 marketed products in pH 1.2, 4.5, and 6.8 according to biowaiver guidelines.

from the criteria already published biowaiver guidelines, the classification would not change with the publication of the ICH guideline. Interestingly, cephalexin monohydrate would be assigned to Class 3 according to the Biopharmaceutics Drug Disposition Classification System (BDDCS), since no metabolism was identified.[108]

In conclusion, cephalexin monohydrate can be considered as a BCS Class 1 drug.

Dissolution

Drugs belonging to BCS class 1 must show a dissolution performance that is either "rapidly dissolving" or "very rapidly dissolving" according to the biowaiver requirements of FDA, WHO, and EMA. Therefore, 85% of the labelled amount of the drug in both the test and reference product must be dissolved in 15 or 30 minutes. In the latter case, an f_2-test must be performed subsequently whereas if both products release the drug within 15 minutes, the f_2-test doesn't have to be applied. Figure 2 demonstrates that the pure drug and both investigated generic products showed "very rapidly" dissolution behavior at pH 1.2 and 6.8. At pH 4.5 however, only the pure cephalexin monohydrate powder was dissolved to an extent of more than 85% in 15 minutes while both marketed products failed to meet the cut-off criteria. After 30 minutes, an average of approximately 14 and 81% cephalexin were dissolved from the products. The very large standard deviations in the dissolution results at pH 4.5 were explained by the failure of the film coating to dissolve, which in turn prevented adequate disintegration during the dissolution test. These observations and results indicate a distinct effect of formulation on the dissolution performance. *In vivo*, however, the poor disintegration/dissolution at pH 4.5 does not seem to be of importance, given that these 2 products both have marketing authorizations for the German market. It seems in this case (as in many others) the comparative dissolution testing according to the biowaiver guidances might be over-discriminating.[109] It might be interesting to apply physiologically based pharmacokinetic (PBPK) modelling to identify a "safe space" for dissolution in this case.

Risk of Bioinequivalence Caused by Manufacturing Variations or Excipients

The solvation state of the cephalexin molecule influences its physicochemical characteristics such as solubility, and thus its pharmacokinetic behavior. Therefore, the influence of environmental parameters such as humidity, temperature or mechanical stress affecting and changing the powder characteristics of cephalexin monohydrate must be considered during the manufacturing process and storage.[20]

Requirements concerning excipients that might be used in generic product formulations of a BCS class 1 drug like cephalexin monohydrate differ depending on the specific biowaiver guideline. The FDA recommends the use of excipients which can currently be found in FDA-approved immediate release solid oral dosage forms. The WHO demands the use of excipients that are also present in the comparator product, while the EMA suggests similar amounts of the same excipients formulated in the reference product. If these recommendations are followed, it should be possible to circumvent any negative influence of excipients on the physiological processes that cannot be simulated by *in vitro* dissolution studies (e.g. effects on motility and permeability).

A single report of an altered intestinal transport of cephalexin in the presence of certain excipients was found in the open literature. The excipient caprylocaproyl macrogolglycerides enhanced the transport of cephalexin in *in vitro* and *in situ* transport experiments.[110] However, such data seems less relevant for a drug like cephalexin, for which a) absorption in humans is largely mediated by active transport, which is not well simulated in such experiments, and b) permeability in humans is already high. Conversely, there have been no literature reports to date of a negative influence on the permeability or the absorption/bioavailability of cephalexin due to excipients.

The lack of reported interactions of cephalexin with other drugs, its high absorption due to active transport, the negligible food effect, the dose-proportionality, and the high number of bioequivalent products on the market, all combine to conclude that effects caused by the listed excipients on the absorption of the drug are minimal.

The dissolution studies revealed problems in the dissolution behavior of the generic products at pH 4.5. Since the respective products are already authorized as marketed generic products and have therefore fulfilled bioequivalence requirements, it can be concluded that the dissolution methods used for BCS-based biowaiver are over-discriminating at pH 4.5 for cephalexin products.

Thus, if the dissolution performance of further generic formulations is found to be similar to that of the reference product, it can be assumed (provided that usual excipients are present in reasonable amounts in both formulations) that the 2 products will be bioequivalent.

To circumvent the over-discrimination at pH 4.5, use of a peak vessel or an increased stirring rate might be useful. Alternatively, there may be an opportunity to explore existing modeling techniques such as PBPK to incorporate formulation effects on *in vitro* dissolution as well as permeability effects (including transporters) into a more holistic description of the *in vivo* performance of cephalexin oral drug products. The model could then be used to develop a reliable "safe space" for the dissolution of the products relative to their *in vivo* performance. Such modeling approaches may provide valuable supporting information, complement biowaiver assessments and avoid ambiguous conclusions; all of which are consistent with the principles laid out in the guidance documents.

Public Health and Patient Risks Associated With Bioinequivalence

For an assessment of the risks following a false positive biowaiver decision, the consequences of sub- or supra-therapeutic levels of cephalexin in patients must be considered. Concentrations below the therapeutic level increase the probability of a therapy failure and an exacerbation of the underlying infection. Cephalexin is recognized as an antibacterial agent and therefore prone to resistance development originating from sub-therapeutic levels. Supra-therapeutic levels on the other hand could lead to adverse effects and toxicity.

Detailed information about the correct choice and use of antibiotics is available. The use of cephalexin monohydrate is only indicated for a few infections caused by specific microorganisms. It may only be applied for a treatment if an infection with these susceptible organisms is proven or at least strongly suspected. A therapy that complies with these instructions minimizes the risk of resistance development and therapy failure. All bioequivalence studies listed in this monograph demonstrated bioequivalence for the examined products and no reports of bioinequivalent cephalexin products were found in the literature. Therefore, the risk of sub-bioequivalent products including cephalexin monohydrate seems to be low.

Cephalexin monohydrate has a wide therapeutic index and is well-tolerated even in higher doses and after repeated administration. The risk of accumulation is comparably low for patients with normal renal function due to the short half-life of the drug. Adverse effects that were observed in clinical studies with different marketed products appear to be moderate, although often reported without details about frequency, and no reports of cephalexin intoxication were located in the open literature. The risk of toxic cephalexin concentrations being reached as a result of an incorrect biowaiver decision therefore appear to be minimal.

Conclusion

Since cephalexin monohydrate is classified as a BCS Class 1 drug, approvals of immediate release solid oral dosage forms of the drug on basis of the BCS-based biowaiver procedure can be recommended in conjunction with the respective guidelines of the FDA, WHO and EMA. Well-established excipients in usual amounts are suggested for the formulation of the generic products. Further, the dissolution test criteria for "rapidly dissolving" or "very rapidly dissolving" must be fulfilled for the test and the comparator product. A drug release of $\geq$85% in 15 minutes or 30 minutes in aqueous media at pH 1.2, 4.5, and 6.8 is required, with an f2-test as an additional requirement if 85% release is achieved within 30 minutes, but not 15 minutes.

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Appendix

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A.1.3. Personal contributions

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

My personal contributions to this publication included the development and validation of HPLC methods for seven of 16 drug substances and the performance of solubility studies for those drugs according to a study protocol of own devising of both first authors; further, the writing of the introduction and materials and methods parts of the paper including the study protocol. The solubility of the other substances was determined by the other first author, Martin Hofsäss, who is the author of the results and discussion part of the publication. The section which discussed degradation challenges and proposed designs of degradation studies was developed together.

Publication 2: Biowaiver monographs for immediate release solid oral dosage forms: proguanil hydrochloride

My personal contributions to this paper consisted of the literature search of the general, physicochemical, and pharmacokinetic properties of the discussed API, a literature search and the assessment of bioequivalence studies of proguanil products, the method development and validation for HPLC analysis, the design and performance of solubility studies, the design and performance of dissolution tests and an UV analysis method, the classification of proguanil hydrochloride to the BCS based on the experimentally obtained solubility data and literature permeability data, the performance of a risk-benefit analysis, and the writing of the manuscript including revisions after the review processes.

Publication 3: Biowaiver monographs for immediate release solid oral dosage forms: cephalexin monohydrate

My personal contributions to this publication included the examination of material that had already been collected in the Institute of Pharmaceutical Technology, an entirely new literature research on the general, physicochemical, and pharmacokinetic properties with the exception of permeability data of cephalexin monohydrate, the search and evaluation of bioequivalence studies that have been conducted with cephalexin products, the adoption and validation of the HPLC method, the design and performance of solubility and degradation studies and dissolution experiments, the classification of cephalexin monohydrate to the BCS based on experimental solubility and literature permeability data, the performance of a risk-benefit analysis, and the writing and revising of the manuscript excepting the results and discussion part regarding permeability.