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USE OF DISSOLUTION DATA IN MODELLING METHODS TO DESCRIBE AND PREDICT PHARMACOKINETICS

Jussi Malkki

DOCTORAL DISSERTATION

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ABSTRACT

The use of in vitro dissolution data to accurately predict the in vivo absorption of biopharmaceuticals has been a goal for decades because it is simpler and more ethically acceptable to conduct in vitro dissolution studies than in vivo bioavailability (BA) or bioequivalence (BE) studies. When developing oral small-molecule drugs, it is critical to understand how changes in formulation may affect drug concentrations in the plasma. Linking the in vitro dissolution profile of drug formulations to the exposures observed in vivo using in vitro-in vivo correlation (IVIVC) models is well suited for this purpose. The biopharmaceutics classification system (BCS) provides a method for small-molecule immediate-release (IR) generic drug development to waive BE studies using only dissolution data. Pharmacokinetic (PK) simulation modelling is an valuable tool for studying IR products that can be accepted as BCS biowaivers. PK models consider the factors influencing the absorption and BA of drugs, including physiological conditions such as pH in the gastrointestinal (GI) tract and the regional distribution of metabolic enzymes and transporters along the GI tract. Moreover, an accurate description of the dissolution and systemic PK processes is required. Physiologically-based pharmacokinetic (PBPK) modelling has recently received considerable attention in biopharmaceutical applications such as constructing IVIVC models and justifying BCS biowaivers. This provides a realistic method to incorporate physiological processes and parameters into the description of the dissolution and PK processes. The characteristics of the different populations can also be easily accounted for.

In this thesis, a level A IVIVC model using a Bayesian approach for a modified-release (MR) formulation series was developed. This model is well suited for waiving BE studies, both in new oral drug formulation development and generic drug development. In addition, PK simulation models were constructed and used to set appropriate and moderately stringent dissolution criteria for BCS class 1 and 3 IR small-molecule drugs. These simulations were performed using numerous parameter combinations obtained from a multidimensional parameter space with a range relevant for actual drugs. In addition, the effect of the MDR-1 efflux transporter on the BE of its substrates was evaluated.

In conclusion, this thesis provides a successful example of using Bayesian methodology to establish a level A IVIVC model for levosimendan. In addition, these findings demonstrate how the regulatory dissolution criteria of BCS class 1 and 3 drugs can be further improved to allow biowaivers when appropriate and require clinical studies only when necessary. The biowaiver criteria for BCS class 1 drugs depend on the T_{max} parameter, and BCS class 3 drugs should be eligible for the biowaiver if the formulations are rapidly dissolved and their dissolution profiles are similar.

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Espoo, December 2023

Jussi Malkki

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I Kortejärvi H, **Malkki J**, Marvola M, Urtti A, Yliperttula M, Pajunen P. Level A in vitro-in vivo correlation (IVIVC) model with Bayesian approach to formulation series. J Pharm Sci 95, 1595-605 (2006)

II Kortejärvi H, Shawahna R, Koski A, **Malkki J**, Ojala K, Yliperttula M. Very rapid dissolution is not needed to guarantee bioequivalence for biopharmaceutics classification system (BCS) I drugs. J Pharm Sci 99, 621-5 (2010)

III Kortejärvi H, **Malkki J**, Shawahna R, Scherrmann JM, Urtti A, Yliperttula M. Pharmacokinetic simulations to explore dissolution criteria of BCS I and III biowaivers with and without MDR-1 efflux transporter. Eur J Pharm Sci 61, 18-26 (2014)

The publications are referred to in the text by their roman numerals.

Author's contribution to the publications included in the doctoral thesis:

Publication I
The author contributed to the experimental design of the study with co-authors. The author constructed all of the Level A IVIVC model with Bayesian approach and did all the evaluation of the model predictability. The author designed and analysed the modelling work with co-authors. The author wrote the manuscript together with Hanna Kortejärvi and other co-authors commented the manuscript. (The author list is presented in alphabetical order, since that was the habit at the Helsinki University of Technology, currently known as Aalto University, except that principal investigator should be the last name. Therefore, this paper should have been as shared first author, since all the data and analysis, and the entire methodology were based on the Master's Thesis of Jussi Malkki).

Publication II
The author contributed to the experimental design of the study with co-authors. The author conducted all the simulations of the work and created the method to present the results graphically. The author designed and analysed the simulation work with co-authors. The author co-wrote manuscript with co-authors.

Publication III
The author contributed to the experimental design of the study with co-authors. The author conducted all of the modelling and simulation work. The author designed and analysed the modelling and simulation work with co-authors. The author wrote the manuscript together with Hanna Kortejärvi and other co-authors commented the manuscript.

LIST OF ADDITIONAL PUBLICATIONS

Additional publications, which are not included in the experimental part of this Thesis are listed below. The author performed part of the simulations, analysed results.

Publications

Margolskee A, Darwich AS, Pepin X, Aarons L, Galetin A, Rostami-Hodjegan A, Carlert S, Hammarberg M, Hilgendorf C, Johansson P, Karlsson E, Murphy D, Tannergren C, Thörn H, Yasin M, Mazuir F, Nicolas O, Ramusovic S, Xu C, Pathak SM, Korjamo T, Laru J, **Malkki J**, Pappinen S, Tuunainen J, Dressman J, Hansmann S, Kostewicz E, He H, Heimbach T, Wu F, Hoft C, Laplanche L, Pang Y, Bolger MB, Huehn E, Lukacova V, Mullin JM, Szeto KX, Costales C, Lin J, McAllister M, Modi S, Rotter C, Varma M, Wong M, Mitra A, Bevernage J, Biewenga J, Van Peer A, Lloyd R, Shardlow C, Langguth P, Mishenzon I, Nguyen MA, Brown J, Lennernäs H, Abrahamsson B. IMI - Oral biopharmaceutics tools project - Evaluation of bottom-up PBPK prediction success part 2: An introduction to the simulation exercise and overview of results. Eur J Pharm Sci 96, 610-625 (2017)

Darwich AS, Margolskee A, Pepin X, Aarons L, Galetin A, Rostami-Hodjegan A, Carlert S, Hammarberg M, Hilgendorf C, Johansson P, Karlsson E, Murphy D, Tannergren C, Thörn H, Yasin M, Mazuir F, Nicolas O, Ramusovic S, Xu C, Pathak SM, Korjamo T, Laru J, **Malkki J**, Pappinen S, Tuunainen J, Dressman J, Hansmann S, Kostewicz E, He H, Heimbach T, Wu F, Hoft C, Pang Y, Bolger MB, Huehn E, Lukacova V, Mullin JM, Szeto KX, Costales C, Lin J, McAllister M, Modi S, Rotter C, Varma M, Wong M, Mitra A, Bevernage J, Biewenga J, Van Peer A, Lloyd R, Shardlow C, Langguth P, Mishenzon I, Nguyen MA, Brown J, Lennernäs H, Abrahamsson B. IMI - Oral biopharmaceutics tools project - Evaluation of bottom-up PBPK prediction success part 3: Identifying gaps in system parameters by analysing In Silico performance across different compound classes. Eur J Pharm Sci 96, 626-642 (2017)

ABBREVIATIONS

ACAT	Advanced compartmental absorption and transit
API	Active pharmaceutical ingredient
AUC	Area under the plasma-concentration curve
BCS	Biopharmaceutics classification system
BDDCS	Biopharmaceutics drug disposition classification system
BA	Bioavailability
BE	Bioequivalence
CaCo-2	Immortalized cell line of human colorectal adenocarcinoma cells
CAT	Compartmental absorption and transit
CL	Clearance
C_{max}	Maximum concentration
CR	Controlled-release
ER	Extended-release
EMA	European Medicines Agency
EMEA	former name of the EMA
FA	Fraction absorbed
Fu	Fraction unbound
FDA	Food and Drug Administration
GI	Gastrointestinal
IR	Immediate-release
IVIVC	In vitro- in vivo correlation
IVIVE	In vitro- in vivo extrapolation
IVIVR	In vitro- in vivo relationship
K10	Elimination rate constant
K_a	Absorption rate constant
MR	Modified-release
PBPK	Physiologically based pharmacokinetics
PBBM	Physiologically based biopharmaceutics modelling
PK	Pharmacokinetics
USP	United States pharmacopoeia
WHO	World Health Organisation

1 INTRODUCTION

Dissolution is the release of active pharmaceutical ingredients (API) from small molecule immediate-release (IR) and modified-release (MR) products. Pharmacokinetic (PK) processes, such as absorption, distribution, metabolism, and excretion, can be used to describe the concentration of a drug in the plasma as a function of time. The use of dissolution data to predict in vivo absorption has been a goal in biopharmaceutics for decades because it is simpler and more ethically acceptable to conduct in vitro dissolution studies than to conduct in vivo bioavailability (BA) or bioequivalence (BE) studies.

During the development of small-molecule drug products, it is important to estimate how changes in formulation affect the in vivo release of drugs and how they are reflected in the concentration-time profile of plasma. In vitro-in vivo correlation (IVIVC) models, where variability is accurately described, are excellent tools. They can be used to study the effects of formulation changes on the in vivo behaviour without conducting in vivo studies for all formulations. Therefore, these models provide an ethical method to aid drug formulation development.

The development of generic drug formulations for small-molecule IR drug products can greatly benefit from biopharmaceutics classification system (BCS) biowaivers which allow the use of dissolution data to waive BE studies. PK simulation models, where physiological conditions affecting absorption, such as pH in the gastrointestinal (GI) tract and regional distribution of metabolic enzymes and transporters along the GI tract, are considered, together with an accurate description of dissolution and systemic PK processes provide a scientifically sound method for studying when IR drug products can be accepted as BCS biowaivers.

The use of physiologically based pharmacokinetic (PBPK) modelling in biopharmaceutical applications, such as constructing IVIVC or justifying BCS biowaivers, has clearly increased in the 2020s (Anand 2022, Heimbach 2021, Han 2022, Wu 2021, Wu 2023b). This increased use is likely due to increased knowledge of the physiological processes and parameters of the GI tract. Although, significant progress has been made in the PBPK modelling of biopharmaceutics for small-molecule IR drug products, there is still work to be done to accurately describe the in vivo release of small-molecule MR drug products and the associated variability physiologically. However, the ability to describe and predict in vivo absorption and concentrations of drugs for different populations, such children, the elderly or people living with diseases, makes PBPK modelling a suitable tool for accurately describing biopharmaceutical processes.

Owing to the long time period over which this thesis was completed, it is structured such that the literature review contains state-of-the-art information until the start of the thesis studies (2005). Research conducted in

the field after the start of this thesis (2005) is presented in the Discussion section. Important papers for the prediction of PK of small-molecule drugs using modelling methods for establishing relationship between dissolution data and in vivo absorption is shown in Table 1.

Table 1. *Important papers for the prediction of PK of small-molecule drugs using modelling methods for establishing relationship between dissolution data and in vivo absorption until 2005.*

Year	Brief description and importance	Full reference
1937	First application of the PBPK model, where drug concentrations were described in human body using five compartments. Concentration-time profiles were solved for each compartment using differential equations. Teorell is considered as father of pharmacokinetics.	Teorell, T. Kinetics of distribution of substances administered to the body. I. The extravascular modes of administration. Arch Int Pharmacodyn et Ther 57, 205-225 (1937).
1964	Description of kinetic models for absorption. Percent absorbed versus time plots were created for different absorption models.	Wagner JG, Nelson E. Kinetic analysis of blood levels and urinary excretion in the absorptive phase after single dose of drug. J Pharm Sci 53, 1392-403 (1964).
1969	First application of the in vitro-in vivo correlation (IVIVC), where percent absorbed in vivo was correlated with percent dissolved in vitro. Percent absorbed was calculated using method described by Wagner and Nelson (1964).	Cressman WA, Janicki CA, Johnson PC, Doluisio JT, Braun GA. In vitro dissolution rates of aminorex dosage forms and their correlation with in vivo availability. J Pharm Sci 58, 1516-20 (1969).
1973	Scale-up of PK between mammalian species including human are thoroughly reviewed. Scaling of individual processes such as physical and chemical processes are discussed using different drugs as examples. Physical processes include such as blood flows, tissue binding, and kidney clearances. Chemical processes include such as metabolic reactions.	Dedrick RL. Animal scale-up. J Pharmacokinet Biopharm 1,435-61 (1973).

Year	Brief description and importance	Full reference
1979	Compartmental representation of the body when describing PK is linked to physiological variables. Link between PK parameters, such as elimination half-life, volume of distribution, clearance, and physiological parameters, such as blood flow, enzyme activity and drug binding, are discussed and illustrated.	Tucker GT. Principles of pharmacokinetics. Ciba Found Symp 74, 13-33 (1979).
1984	The mixing tank model for simulating GI absorption was introduced. The effect of drug parameters, such as pK_a, solubility, and intrinsic wall permeability, and system parameters ,such as pH profile, volume of intestinal contents, and intestinal flow rate, on drug absorption were studied by performing simulations. It was concluded that simulation results were consistent with observed data.	Dressman JB, Fleisher D, Amidon GL. Physicochemical model for dose-dependent drug absorption. J Pharm Sci 73, 1274-9 (1984).
1985	Thorough review of physiological PK models and interanimal species scaling. Basic principles and development of these methods are clearly illustrated.	Rowland M. Physiologic pharmacokinetic models and interanimal species scaling. Pharmacol Ther 29, 49-68 (1985).
1989	These two papers were published around the same time and introduced a Caco-2 model, This in vitro cell line can be used to study intestinal permeability. Major advantages of this method are that cells are of human origin and they allow clearly longer duration of in vitro experiments than earlier methods.	Hidalgo IJ, Raub TJ, Borchardt RT. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. Gastroenterology 96, 736-49 (1989).
1990		Artursson P. Epithelial transport of drugs in cell culture. I: A model for studying the passive diffusion of drugs over intestinal absorptive (Caco-2) cells. J Pharm Sci 79, 476-82 (1990).

Year	Brief description and importance	Full reference
1992	Human in vivo model for studying jejunal perfusion was introduced which also allowed studying absorption of different regional segments of the intestine.	Lennernäs H, Ahrenstedt O, Hällgren R, Knutson L, Ryde M, Paalzow LK. Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. Pharm Res 9, 1243-51 (1992).
1995	Introduction of biopharmaceutics classification system (BCS), where drugs can be classified into four classes based on their dissolution and gastrointestinal permeability. This system allows setting standards for in vitro dissolution testing that correlate with in vivo absorption.	Amidon GL, Lennernäs H, Shah VP, Crison JR. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm Res 12, 413-20 (1995).
1996	Compartmental Absorption Transit (CAT) model was presented for description of the transit process of oral dosage forms through the human small intestinal tract. Profile of small intestinal transit flow was captured by compartmental transit model with seven compartments. This model was found superior to single compartment model and less complex than dispersion model.	Yu LX, Crison JR, Amidon GL. Compartmental transit and dispersion model analysis of small intestinal transit flow in humans. Int J Pharm 140, 111-18 (1996).
1997	First guideline drafted by FDA on how to establish IVIVC for oral dosage forms	FDA. Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations. Guidance for industry (1997).
2001	Development of CAT model to advanced compartmental absorption and transit (ACAT) model. The rate, extent, and location of drug liberation for controlled-release drug products, dissolution, passive and active absorption, and saturable metabolism are described. Fraction absorbed, bioavailability, and concentration-time profiles are successfully predicted for common drugs using ACAT model.	Agoram B, Woltosz WS, Bolger MB. Predicting the impact of physiological and biochemical processes on oral drug bioavailability. Adv Drug Deliv Rev 50 Suppl 1, S41-67 (2001).

Year	Brief description and importance	Full reference
2004	Thorough description and possibilities of in silico simulations using in vitro metabolic drug-drug interaction data for prediction of in vivo situation.	Rostami-Hodjegan A, Tucker G. 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions. Drug Discov Today Technol 1, 441-8 (2004).
2005	Introduction of refined version of BCS named biopharmaceutics drug disposition classification system (BDDCS) where routes of drug elimination and the effects of efflux and absorptive transporters on drug absorption is also considered.	Wu CY, Benet LZ. Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res 22, 11-23 (2005).
2005	First biowaiver introduced for BCS class 3 drug (ranitidine). Previously, biowaivers were only proposed for BCS class 1 drugs.	Kortejärvi H, Yliperttula M, Dressman JB, Junginger HE, Midha KK, Shah VP, Barends DM. Biowaiver monographs for immediate release solid oral dosage forms: ranitidine hydrochloride. J Pharm Sci 94, 1617-25 (2005).
2005	The first IVIVC constructed using biorelevant dissolution media.	Sunesen VH, Pedersen BL, Kristensen HG, Müllertz A. In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. Eur J Pharm Sci 24, 305-13 (2005).

2 REVIEW OF THE LITERATURE UNTIL 2005

2.1 LINKING DRUG DISSOLUTION WITH IN VIVO EXPOSURE

The ability to predict in vivo exposure using in vitro dissolution data would enable a decrease in the number of clinical studies and lessen the time needed for drug development. To link in vitro dissolution and in vivo exposure, the factors that influence dissolution and absorption in the GI tract need to be understood. Several modelling methods have been proposed to correlate the in vitro-determined dissolution of drug products and the in vivo absorption profiles of oral small-molecule drugs. The goal of these methods is to use in vitro-generated data to waive and better understand in vivo BA/BE studies. These methods include IVIVR/IVIVC, BCS and PBPK models. IVIVR/IVIVC and PBPK models can be written using general modelling software or one can use customised software. An accurate description of variability is important when describing and predicting in vivo exposure at the population level. Nonlinear mixed-effects modelling and the Bayesian approach are excellent tools for this purpose.

2.1.1 DISSOLUTION

Dissolution is a process in which a drug substance is released and dissolved from the drug product into liquids in the GI tract (Rowland 1995). The drug can only be absorbed after dissolution. Dissolution can be studied in vitro by measuring the percentage of the drug dissolved from the dosage form. Authorities such as the Food and Drug Administration (FDA) have clear requirements for dissolution testing (FDA Guidance 1997a). Dissolution testing conditions such as apparatus, rotation speed, temperature, dissolution medium, dissolution volume, and sampling schedule were clearly defined. Differences in the testing conditions leads to differences in the observed dissolution data. As a result of in vitro dissolution test one obtains dissolution profile as a function of time. The dissolution profiles vary greatly between the IR and MR products. FDA has defined that 80% of IR solid oral drug products containing a highly soluble drug substance should have dissolved within 30 min (FDA Guidance 2018). If the dissolution is slower than this criterion, then product is considered to have an MR profile.

2.1.2 ABSORPTION FROM GI TRACT

For an orally administered drug to be absorbed, it must be dissolved (Rowland 1995). In addition to dissolution, drugs face several barriers during absorption. These processes include excretion, metabolism, permeation and they have been depicted in Figure 1. The intestinal wall contains several absorptive (such as MCT1 and PEPT1) and efflux (such as MDR1 and BCRP) transporters in the apical and basolateral membranes (Pang 2003). Absorptive transporters increase and efflux transporters decrease permeation. The gut wall also contains several drug-metabolising enzymes, such as CYP and UGT.

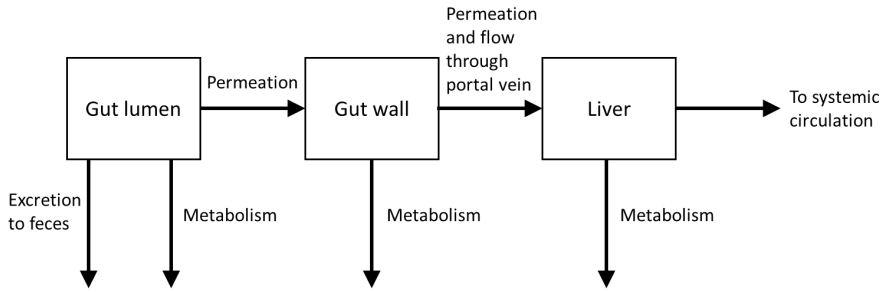


Figure 1 The main barriers and processes involved in drug absorption from the GI tract, figure is modified from Rowland (2005).

The total BA (F) is the overall fraction of the drug reaching systemic circulation and can be calculated as follows

$$F = F_F \cdot F_G \cdot F_H, \quad (1)$$

where F_F is the fraction which is not excreted in the faeces or metabolised in the gut lumen, F_G is the fraction that escape metabolism in the gut wall, and F_H is the fraction that escape liver metabolism (Rowland 2005).

2.1.3 IVIVC

In vitro dissolution rates of sustained-release formulations can be correlated with in vivo absorption (=currently BA) profiles (Cressman 1969). To perform a correlation, the plasma concentration-time level of the drug should be converted to a percentage of the dose absorbed as a function of time using a method called deconvolution. This can be accomplished using the method developed by Wagner and Nelson (1964). In this method, the following equation was used to estimate the percentage absorbed at a given time:

$$\%absorbed = \frac{A_T}{A_\infty} \cdot 100 = \frac{C_T + K \cdot \int_{t=0}^{t=T} C dt}{K \cdot \int_{t=0}^{t=\infty} C dt} \cdot 100, \quad (2)$$

where A_T is cumulative amount of drug absorbed from time 0 to time T, A_∞ is the amount of drug eventually absorbed, K is overall elimination rate constant and C_T is plasma concentration in time T. IVIVC can be defined as a predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, such as plasma drug concentration or amount of drug absorbed (FDA Guidance 1997b). A representative example of the in vitro-in vivo correlation between the percentage of dissolved dose and the percentage of dose absorbed using deconvolution is illustrated in Figure 2. The correlation between in vitro dissolution and in vivo absorption can also be established using convolution which directly relates in vitro dissolution rates to in vivo plasma concentration profiles (Buchwald 2003). This can be achieved with compartmental models where the rate of in vitro dissolution is connected to the rate of in vivo input through a general function where lag time and scaling are possible. This is illustrated in Figure 3.

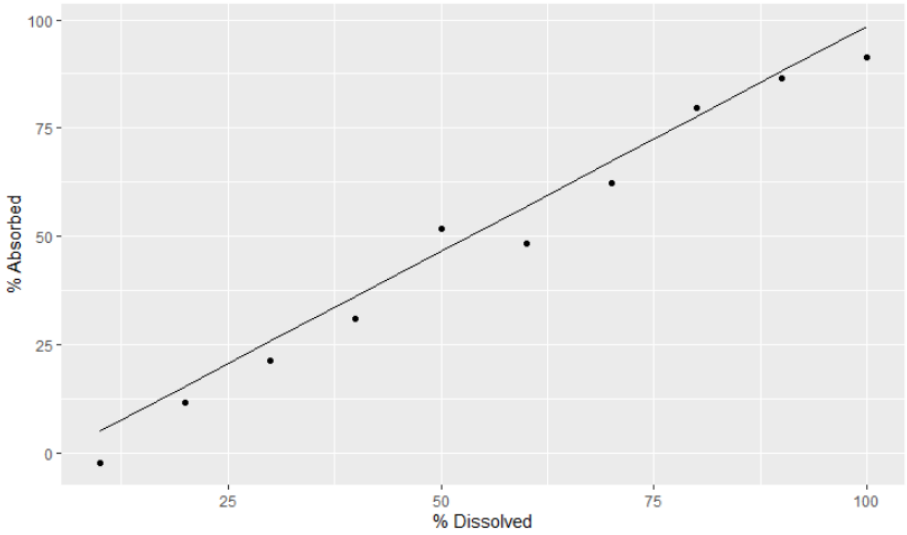


Figure 2 Representative example of quantitative IVIVC of sustained-release formulations using deconvolution.

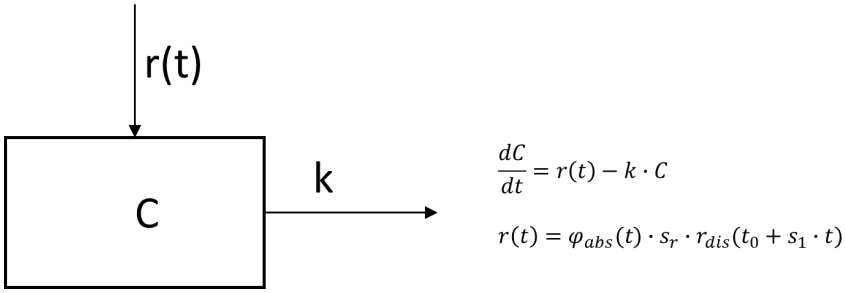


Figure 3 An example of a direct convolution-type one-compartment IVIVC model where C is the plasma concentration, k is the elimination rate constant, r(t) is the rate of in vivo input, $r_{dis}(t)$ is the in vitro dissolution rate, $\varphi_{abs}(t)$ is the time-dependent multiplying factor, t_0 is the lag time, and s_r and s_1 are the scaling constants. Figure modified from Buchwald (2003).

The goal of this study was to accurately and precisely predict the expected in vivo absorption characteristics of an extended-release (ER) product from its dissolution profile. If in vitro dissolution is predictive of in vivo absorption, the established relationship could allow the introduction of changes in formulations and manufacturing without conducting an in vivo BA/BE study. Three levels of IVIVC have been defined by the authorities (FDA, 1997; EMA, 1999):

- Level A is typically linear and represents a point-to-point relationship between the in vitro dissolution and in vivo input rate.
- Level B uses the principles of statistical moment analysis where the mean in vitro dissolution time is compared with either the mean residence time (MRT) or the mean in vivo dissolution time.
- Level C is a single-point relationship between dissolution parameters (such as percentage dissolved in 4 h) and PK parameters, such as the area under curve (AUC).

Although IVIVC can be defined using two formulations with different release profiles, the FDA recommends that three or more formulations with different release rates should be used to develop IVIVC (FDA 1997). To use the developed IVIVC model for waiving in vivo study, the predictability of the established correlation should be evaluated. Predictive ability should be evaluated both internally and externally using data used to develop the model and using data not used in the development of the IVIVC. Assessing predictability with an appropriate metric is a crucial part of the IVIVC development and should not be overlooked (Eddington et al. 1998). Inter- and intrasubject variability in PK can be greater than the variability between formulations (Mauger et al 1997). Methods to describe variability in IVIVC models exist; for example using nonlinear mixed effects modelling, as was done by Bigora et al. (1997). The authors first calculated the in vivo cumulative

amount of drug absorbed by ER formulations by performing deconvolution using IR formulation data as a unit impulse function. The correlation between the in vivo cumulative amount absorbed and the in vitro cumulative amount dissolved was modelled with linear and four nonlinear relationship equations using nonlinear mixed effects modelling to simultaneously estimate the parameters and their intersubject variability. Nonlinear mixed-effects modelling is suitable for describing variability when developing an IVIVC. Although nonlinear relationships describe the data better than linear relationship in this example, the use of more complex nonlinear functions should be carefully evaluated.

2.1.4 BCS

Classifying orally administered drugs into classes based on their biopharmaceutical characteristics will make it possible to set standards for in vitro dissolution testing which will correlate with in vivo processes (Amidon et al 1995). BCS is based on the concept that the fundamental parameters controlling the rate and extent of absorption are dissolution and GI permeability. Drugs can be divided into four classes based on their solubility and permeability. This classification is illustrated in Figure 4. There are differences between these classes regardless of whether an IVIVC is expected. For low permeability classes, limited or no IVIVC was expected. For class 1 drugs correlation between in vitro dissolution and in vivo absorption is possible if the dissolution rate is slower than gastric emptying rate. The possibility of IVIVC was the highest for class 2 drugs. 130 orally administered drugs from the Model list of Essential Medicines of the World Health Organisation was attempted to classify based on BCS (Lindenberg et al 2004). Only 61 of the 130 drugs were classified into the four classes with certainty. The remaining 79 drugs were not assigned to specific classes mainly because of insufficient or conflicting data.

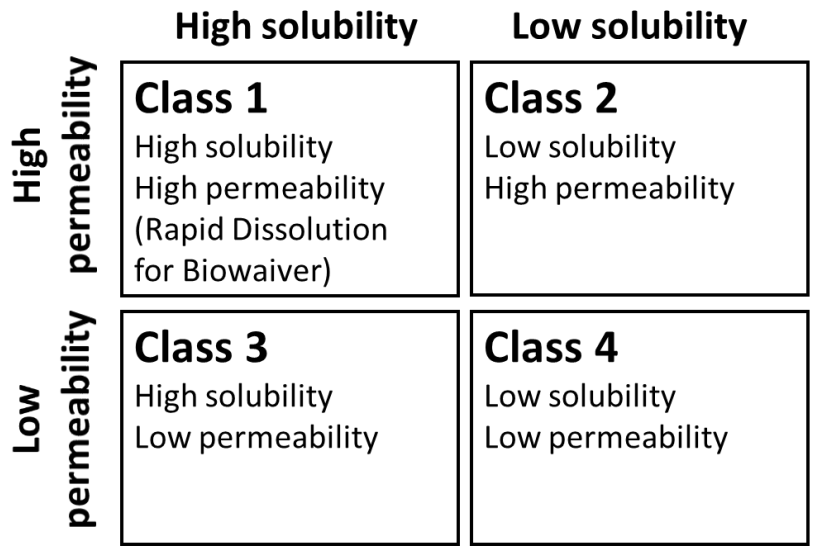


Figure 4 The BCS as defined by the FDA (Guidance 2000) after Amidon 1995, modified from Wu 2005.

The BCS states that if an IR drug (BCS class 1) has high solubility, high permeability and product dissolves rapidly, a waiver for performing BE studies (biowaiver) can be scientifically justified. Biowaiver monographs were written for the class 1 compounds verapamil hydrochloride, propranolol hydrochloride and atenolol based on literature data (Vogelpoel et al 2004). The purpose of these monographs is to provide a scientific basis for biowaivers based on the BCS using literature data. Although limited IVIVC is expected, a waiver of BA/BE studies has been proposed for class 3 compounds for fast-dissolving products without excipients that may modify GI transit or membrane permeation (Blume 1999). The first biowaiver monograph of class 3 compounds was presented for ranitidine hydrochloride (Kortejärvi 2004). It stated that ranitidine hydrochloride could be granted a biowaiver under conditions where the IR solid oral dosage form of ranitidine hydrochloride is formulated with a certain list of excipients in typical amounts and the product is rapidly dissolving.

2.1.5 BDDCS

Modification of the BCS of orally administered drugs was suggested where overall drug disposition is predicted with consideration of routes of drug elimination and the effects of efflux and absorptive transporters on drug absorption (Wu et al 2005). This system considers when the transporter-

enzyme interplay will yield clinically significant effects (such as low BA and drug-drug interactions), the direction, mechanism, and importance of food effects; and transporter effects on postabsorption systemic drug concentrations following oral and intravenous dosing. Compounds with high permeability are good candidates for cytochrome P450 (CYP) enzymes (Smith 1994). Analyses of drugs in the four BCS classes showed that compounds in classes 1 and 2 were primarily eliminated through metabolism whereas class 3 and 4 compounds were primarily excreted unchanged in the urine and bile (Wu et al 2005). Therefore, it was suggested that the permeability component in the original BCS is changed to a route of elimination in BDDCS. This modification was expected to expand the number of class 1 drugs eligible for a waiver and it was also expected to provide enhanced predictability of drug disposition for drugs in classes 2, 3 and 4. The modified classification system is illustrated in Figure 5.

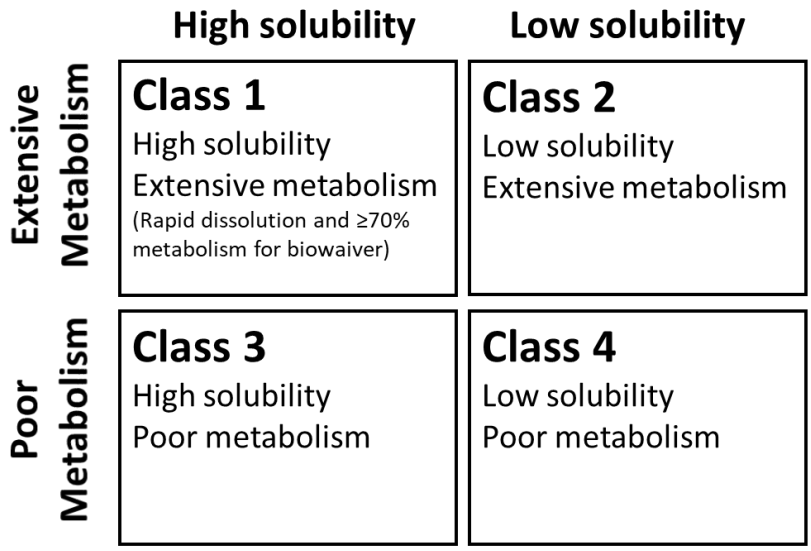


Figure 5 The BDDCS, modified from Wu and Benet (2005).

2.1.6 PBPK MODELLING

In PBPK modelling, PK processes are generally defined in terms of parameters related to the underlying physiology, anatomy and biochemistry (Rowland 1985). PK events in each organ, tissue, or group of kinetically related tissues, can be described using tissue size, vascular perfusion, permeability of the tissue membrane to the drug, binding or partitioning of the drug between

components in the blood and those in the tissue, and elimination processes. The PBPK concepts are described in more detail in Section 2.2.

2.1.7 NONLINEAR MIXED-EFFECTS MODELLING

Nonlinear mixed effects modelling is a method for describing variability, for example, in drug concentration-time data. The nonlinear mixed-effects model is a hierarchical model which typically contains two levels of random effects to describe between subject parameter variability and residual variability in observations that are not accounted for in other parts of the model (Pillai 2005). The nonlinear mixed-effects model can be described using the following equations for the structural model (3), random effects, and covariate model (4):

$$y_{ij} = f(x_{ij}, \varphi_i) + \varepsilon_{ij} \quad \varepsilon_{ij} \sim N(0, \sigma^2) \tag{3}$$

$$\varphi_i = g(z_i, \theta) + \eta_i \quad \eta_i \sim N(0, \Omega), \tag{4}$$

where y_{ij} is jth observation in ith subject, f is parametric function of the structural model, x_{ij} is design variables for the jth observation in the ith subject, φ_i is model parameters for ith subject, ε_{ij} is residual error for the jth observation in ith subject, σ^2 is variance of the residual unidentified variability, g is parametric function of the covariate model (reduces to θ if no covariates are included), z_i is vector of covariates for the ith subject, θ is vector of fixed effects parameters, η_i is vector of individual random effects and Ω is variance-covariance matrix of the random effects parameters.

2.1.8 BAYESIAN DATA ANALYSIS

Bayesian data analysis can be considered a practical method for making inferences from data using probability models for observed and unobserved quantities (Gelman 1995). For a problem where observed data were y and model parameters were θ posterior distribution of θ given y can be described as follows using Bayes's rule

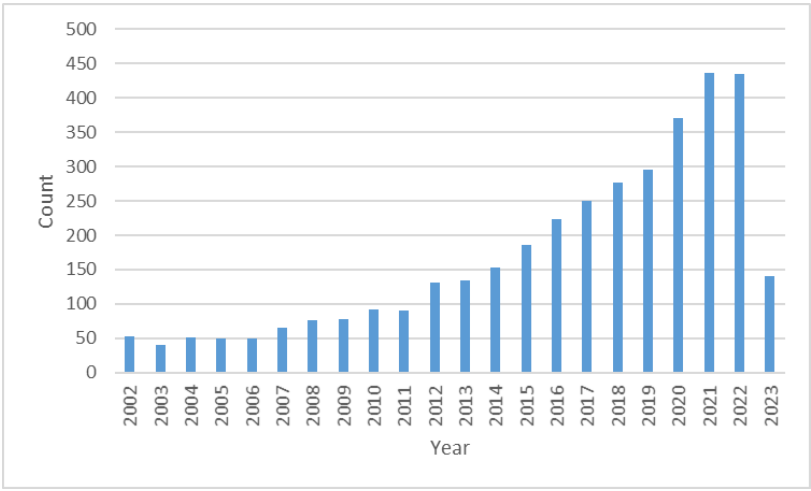
$$p(\theta|y) = \frac{p(\theta)p(y|\theta)}{p(y)}, \tag{5}$$

which can be presented as unnormalised posterior distribution as follows

$$p(\theta|y) \propto p(\theta)p(y|\theta) \tag{6}$$

where p(θ) is prior distribution and p(y| θ) is sampling distribution.

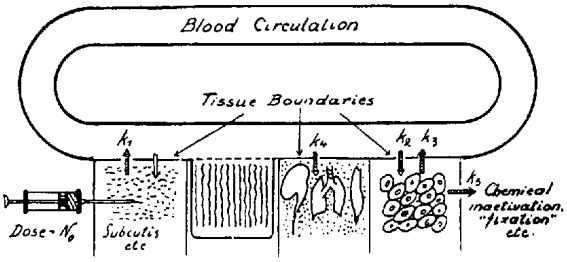
2.2 DEVELOPMENT OF PBPK



Graphic 1 Number of publications per year containing key word “pbpk” from 2002 until 4.4.2023 in PubMed.

2.2.1 HISTORY OF PBPK

PK aim to provide a mathematical presentation for describing and predicting the concentration of drugs in the body as a function of time (Tucker 1979). The PK principles are often explained by the compartmental representation of the body. These compartments can be purely hypothetical with no relationship to real tissues or organs. However, in the context of physiological modelling, circulating blood and individual tissues can be assigned to their own compartments, in which physiological and anatomical properties are considered (Rowland 1985). PK parameters such as elimination half-life, volume of distribution and clearance are easily understood when they are directly related to primary physiological parameters such as blood flow, enzyme activity and drug binding (Tucker 1979). In 1937, the essential features of a physiological model, such as individual tissues and circulating blood, were introduced within the context of a more general model describing drug kinetics (Teorell 1937). The first physiological model includes five compartments, as shown in Figure 6.



Local	Drug depot	Blood + equivalent Blood vol	Kidney etc elimination	Tissues	Tissue inactivation
Symbol	D	B	K	T	I
Amount	x	y	u	z	w
Volume	V_1	V_2	—	V_3	—
Concentration	x/V_1	y/V_2	—	z/V_3	—
Perm. coeff	k_1'	—	k_4'	k_2'	—
Velocity constant out	$k_1 = k_1'/V_1$	—	$k_4 = k_4'/V_2$	$k_3 = k_2'/V_2$	k_5
Velocity constant in	neglected	—	not existing	$k_2 = k_2'/V_2$	—
Name of process	Resorption	—	Elimination	Tissue take up -"- output	Inactivation

FIG. 1

Scheme of the Concept of Drug Distribution used in this paper.
Instead the *injection* pictured in the figure, the administration of the drug depot can be made *per os*, *per rectum*, *by inhalation*, etc.

Figure 6 Scheme of the first physiologic PK model by Teorell (1937)

Physiological modelling of anaesthetic agents was considered in the 1950s and the 1960s (Papper 1963). Since then, physiological modelling has been used for several other drugs (Dedrick 1973, Himmelstein 1979). For example, drugs such as thiopental, methotrexate, cytarabine, adriamycin, cyclocytidine and digoxin have been described using PBPK models (Bischoff 1968, Bischoff 1970, Bischoff 1971, Dedrick 1972, Harris 1975, Himmelstein 1977, Harrison 1977). Physiological modelling methods have also been applied to environmental agents (Andersen 1982). The use of PBPK has increased, as is evident from the number of publications shown in Graphic 1.

2.2.2 METHODOLOGY

PBPK models can be described using three main components: physiological data, drug-specific data and model structure (Rowland 2004). Physiological parameters characterise the anatomical structures and physiological processes of animals and humans (Nestorov 2003). These parameters include body weight; tissue/organ/fluid weights and volumes; volumes of different tissue sections, such as tissue vascular, interstitial, and cellular volumes; cardiac output; regional and tissue blood flows; bile and lymph flows; and other allometric parameters (=changes with body size). Drug-specific parameters include parameters characterising processes such as binding (such as fraction

unbound in blood, plasma or tissues), partitioning (such as blood:plasma ratio or tissue:plasma distribution coefficients), permeability (such as permeability surface area products), and metabolism (intrinsic clearance).
The model structure comprises tissues/organs connected by blood flow. An example of the model structure is shown in Figure 7.

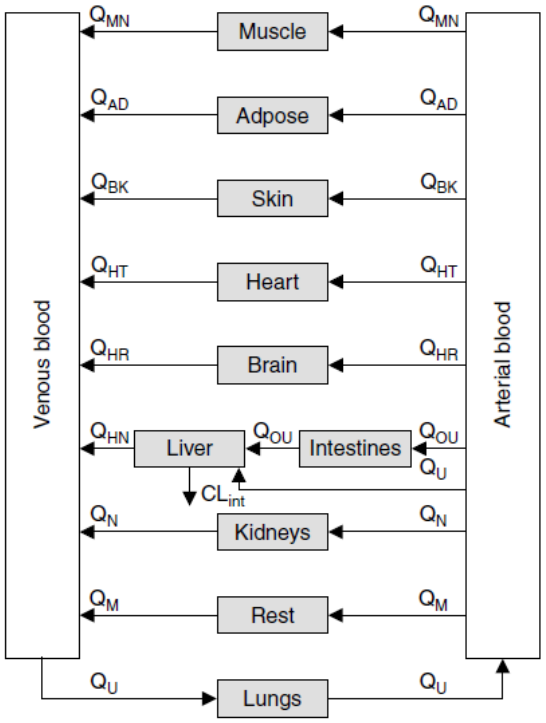


Figure 7 PBPK model structure, where CL_{int} = intrinsic clearance; Q = blood flow (Nestorov 2003), and the subscripts for Q indicate blood flow to specific tissues.

Tissues can be perfusion rate-limited or permeability rate-limited. The perfusion rate-limited tissue model represents the tissue as a single well-stirred compartment, whereas the permeability rate-limited tissue model represents the tissue as two or more well-stirred compartments with permeability rate-limited transfer between them. The different tissue models are shown in Figure 8.

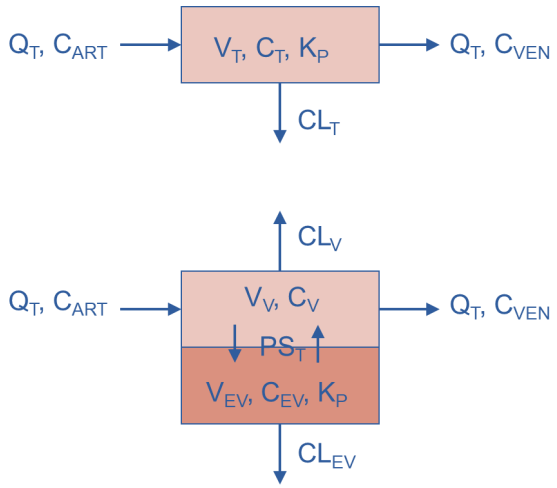


Figure 8 In the top perfusion rate-limited tissue model and the bottom permeability rate-limited tissue model, C is concentration, CL is clearance, K_P is tissue:plasma distribution coefficient, PS is permeability surface area coefficient, Q is blood flow, V is volume, subscript ART is arterial, EV is extravascular, T is tissue, V is vascular, and VEN is venous (Nestorov 1998).

PBPK models are often described using ordinary differential equations (Teorell 1937, Himmelstein 1979). Differential equations were specified for each compartment. Equations differ for different types of tissues, for example whether the tissue is an eliminating organ (Rowland 1986). PBPK models can first be constructed for animal species such as rats and then scaled to humans (Bernareggi 1991). An example of a set of ordinary differential equations describing the PBPK model for cyclosporins is presented below:

Blood-to-plasma concentration ratio in effluent blood:

$$R_i = \frac{C_b}{C_p} = 1 - H + H \cdot fu + \frac{H \cdot fu(n \cdot P_T)}{Cu_{50} + \frac{C_{T,i}}{Kpu_i}}, \quad (7)$$

where R_i is blood-to-plasma concentration ratio in venous blood leaving the ith organ, H is haematocrit, fu is fraction unbound in plasma,$n \cdot P_T$ is the maximum capacity of the erythrocytes for binding cyclosporine, Cu_{50} is the unbound plasma concentration at 50% of maximum binding capacity, $C_{T,i}$ is concentration in ith tissue and Kpu_i is tissue-to-unbound plasma concentration ratio at steady state for ith tissue.

Noneliminating organs and tissues:

$$V_{T,i} \frac{dC_{T,i}}{dt} = Q_{T,i} \left(C_A - \frac{C_{T,i} \cdot R_i}{fu \cdot Kpu_i} \right), \quad (8)$$

where $V_{T,I}$ is the volume of the ith tissue, $Q_{T,I}$ is the blood flow for the ith tissue and C_A is the arterial blood concentration.

Liver:

$$V_{LI}\frac{dC_{LI}}{dt} = \sum_i \frac{Q_{T,i}\cdot C_{T,i}\cdot R_i}{fu\cdot Kpu_i} + Q_{LI}\cdot C_A - \frac{C_{LI}}{Kpu_{LI}}\left(\frac{Q_H\cdot R_{LI}}{fu} + CL_{int}\right), \quad (9)$$

where LI is the liver; i includes the gut, spleen, stomach and pancreas; Q_H is the hepatic blood flow and CL_{int} is the intrinsic clearance calculated as

$$CL_{int} = \frac{Q_H\cdot E}{fu_b\cdot(1-E)}, \quad (10)$$

where E is CL_b/Q_H, fu_b is the unbound fraction in the blood and CL_b is clearance from the blood.

Venous blood:

$$V_V\frac{dC_V}{dt} = \sum_i \frac{Q_{T,i}\cdot C_{T,i}\cdot R_i}{fu\cdot Kpu_i} - Q_1\cdot C_V + R_0, \quad (11)$$

where i includes the brain, heart, kidneys, skeleton, testes, muscle, liver, skin, and adipose tissue; V_v is the volume of the venous blood pool, C_V is the mixed venous blood concentration, R_o is the dose/duration of the infusion; and Q_1 is the total venous blood flow (cardiac output).

Lungs:

$$V_1\frac{dC_1}{dt} = Q_1\left(C_V - \frac{C_1\cdot R_1}{fu\cdot Kpu_1}\right), \quad (12)$$

where V_1 is the lung volume, C_1 is the concentration in the lungs, and R_1 is the blood-to-plasma ratio in the lungs.

Arterial blood:

$$V_A\frac{dC_A}{dt} = Q_1\left(\frac{C_1\cdot R_1}{fu\cdot Kpu_i} - C_A\right), \quad (13)$$

where V_A is volume of arterial blood pool.

Systemic blood concentration:

$$C_p = \frac{C_b}{1-H+H\cdot fu+H\cdot fu(n\cdot P_T)/(Cu_{50}+fu\cdot C_p)}. \quad (14)$$

2.2.3 DESCRIPTION OF ADME PROCESSES IN PBPK MODELS

The main PK processes involved include absorption, distribution, metabolism, and excretion. In this chapter, absorption, distribution and metabolism in PBPK modelling are explained in detail because their role is greater than excretion in the context of this thesis.

2.2.3.1 Absorption

Several parameters are involved in the complex process of drug absorption in the GI tract (Agoram 2001, Nestorov 2003). Drug- or drug -product-specific parameters of drug absorption include pK_a, solubility, stability, diffusivity, lipophilicity, salt form, surface area, particle size, crystal form, release and dissolution rates of dosage forms (solution, tablet, capsule, suspension, emulsion, gel and modified release), permeability across the intestinal wall, and intrinsic metabolic clearance in the gut or liver. Physiological parameters involved in absorption include GI pH, rate of gastric emptying, transit times in the small and large bowel, efflux and transport protein expression levels, and the number of metabolic enzymes in gut and liver.

The regional permeability (currently absorption) of drugs can be studied using an in vivo model (Lennernäs 1992). In this invasive model two inflatable balloons are used to isolate a region of the GI tract, and the perfusion of the drug in that region from the apical to the basolateral side can be determined. The active and passive permeability of drugs in the intestine can also be studied using an in vitro CaCo-2 model (Hidalgo 1989, Artursson 1990). CaCo-2 cells are well-differentiated human colon adenocarcinoma cells. A comparison of the above-mentioned in vivo and in vitro methods was summarized by Lennernäs (1997). It was observed that for passively transported drugs, permeability could be predicted well using all methods. For drugs with carrier-mediated transport mechanisms special care is needed when interpreting the results and especially with preclinical permeability models a scaling factor must be used.

To simulate the conditions in the lumen of the GI tract as realistically as possible, a multi-compartmental GI model was designed (Minekus et al. 1995). Several mathematical models have been developed to simulate drug absorption (Suzuki 1970a, Suzuki 1970b, Ho 1972, Dressman 1984, Yu 1996, Yu 1999, Agoram 2001). Earlier models described drug absorption theoretically using a two-phase compartment model with a well-stirred aqueous phase, an aqueous diffusion layer, and a lipid barrier. In the mixing tank model of Dressman (1984), transit within intestinal tract was also described using two separate tanks. In the compartmental absorption transit model proposed by Yu et al., transit across the intestinal tract included seven different compartments. The advanced compartmental absorption transit (ACAT) model includes the key processes of oral drug absorption: release, dissolution, luminal degradation, metabolism and absorption/exsorption of drugs as they transition through successive compartments (Agoram 2001).

The compartments and processes involved in the ACAT model are shown in Figure 9.

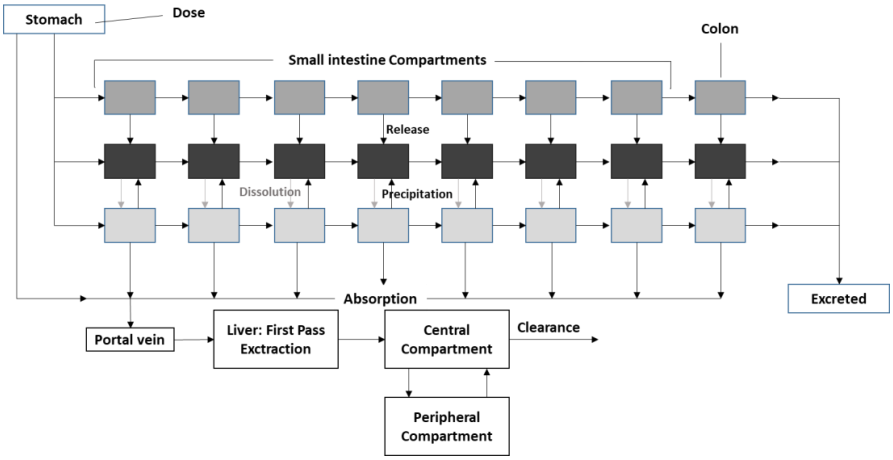


Figure 9 ACAT model (Agoram 2001).

The ACAT model was first implemented into PBPK modelling software Gastroplus™.

2.2.3.2 Distribution

The distribution of drugs from the plasma into tissues can be described using tissue:plasma partition coefficients which are estimated using in vitro/in silico data (Poulin 2000, Poulin 2001, Rodgers 2005, Rodgers 2006). An alternative method for studying tissue:plasma partitioning using nonclinical in vivo methods where the drug would need to be in a steady -state, would require a lot of time and would be rather expensive. The parameters needed to estimate these partition coefficients include the partition coefficient between vegetable oil and water or if not available, the partition coefficient between octanol and water (log P, currently the standard method); unbound fraction of drug in plasma and in tissues; fractional volumes of water, neutral lipids, and phospholipids in plasma and tissues; fractional content of plasma in blood; and fractional content of interstitial space in tissues. The equations described by Poulin used to calculate tissue:plasma partition coefficients are shown below (Poulin 2000, Poulin 2001).

Non-adipose tissue assuming homogenous distribution and passive diffusion:

$$P_{t:p} = \frac{K_{vo:w}\cdot(V_{nt}+0.3\cdot V_{pht})+(V_{wt}+0.7\cdot V_{pht})}{K_{vo:w}\cdot(V_{np}+0.3\cdot V_{php})+(V_{wp}+0.7\cdot V_{php})}\cdot\frac{fu_p}{fu_t} \quad (15)$$

Non-adipose tissue where distribution is restricted to intestinal space in tissues:

$$P_{t:p} = \frac{F_t}{F_p} \cdot \frac{fu_p}{fu_t} \quad (16)$$

Adipose tissue:

$$P_{at:p} = \frac{K^*_{vo:w} \cdot (V_{nt} + 0.3 \cdot V_{pht}) + (V_{wt} + 0.7 \cdot V_{pht})}{K^*_{vo:w} \cdot (V_{np} + 0.3 \cdot V_{php}) + (V_{wp} + 0.7 \cdot V_{php})} \cdot \frac{fu_p}{1} \quad (17)$$

where $K_{vo:w}$ is the vegetable oil:water partition coefficient (can be predicted using $P_{o:w}$ (Leo 1971)), $K^*_{vo:w}$ is the vegetable oil:water partition coefficient representing the distribution between non-ionised species in oily phase and non-ionised and ionized species in aqueous phase, fu_p is the unbound fraction of drug in the plasma, fu_t is the unbound fraction of drug in tissues (can be predicted using fu_p), V_{nt} is the fraction of total volume of neutral lipids in tissue, V_{pht} is the fraction of total volume of phospholipids in tissue, V_{wt} is the fraction total volume of water in tissue, V_{np} is the fraction of total volume of neutral lipids in plasma, V_{php} is the fraction of total volume of phospholipids in plasma, V_{wp} is the fraction of total volume of water in plasma, F_t is the fractional content of interstitial space in tissues, and F_p is the fractional content of plasma in blood.

2.2.3.3 Gut and liver metabolism

The gut wall and liver are the two main places for drug metabolism. One method to estimate the fraction of drug escaping the gut metabolism (F_{Gut}) is to describe it using the following equation (Rostami-Hodjegan 2004)

$$F_{Gut} = \frac{Q_{Gut}}{Q_{Gut} + fu_{Gut} \cdot CL_{int,Gut}}, \quad (18)$$

where Q_{Gut} is the nominal blood flow in the gut, fu_{Gut} is the free fraction of the drug at the enzyme site in the gut and $CL_{int,gut}$ is the intrinsic metabolic clearance in the gut.

Hepatic metabolism can be calculated using different models such as the well-stirred, parallel-tube and dispersion models (Pang 1977, Roberts 1986, Rowland 1973, Wilkinson 1975). The most commonly used model is the well-stirred model presented below:

$$CL_H = \frac{Q_H \cdot fu_b \cdot CL_{int}}{Q_H + fu_b \cdot CL_{int}}, \quad (19)$$

where CL_H is the hepatic clearance, Q_H is the liver blood flow, fu_b is the fraction unbound in blood and CL_{int} is the intrinsic clearance.
Intrinsic clearance is the ability of an organ (such as gut or liver) to metabolise drugs without restrictions such as blood protein binding or blood flow to organs (Rostami-Hodjegan 2004). Intrinsic clearance can be determined using different in vitro systems such as microsomes, hepatocytes or recombinant CYP enzymes (McGinnity 2001).

2.2.4 SOFTWARE

There is a wide variety of general software for coding, developing and implementing PBPK models such as ACSL (with SIMUSOLVE), MATLAB with SIMULINK, Stella, Berkeley Madonna, Mathematica, CMATRIX, SCoP and SCoPfit programs. There are also specialised software for PBPK modelling such as Gastroplus, IDEA, SIMCYP, and PK-Sim (Agoram 2001, Parrott 2002, Rostami-Hodjegan 2004, Willmann 2003). These specialised software packages include model structure, drug-specific and physiological parameters. Short descriptions of these specialized software are included Table 2.

Table 2. *Short descriptions of specialized software used for PBPK modelling*

Software	Short description
Gastroplus	Simulates GI absorption and PK of drugs, is originally based on CAT model (Yu 1996) with nine compartments for the digestive tract (stomach, seven small intestinal compartments, and colon).
IDEA	Simulates human physiology and accounts for regional variations in intestinal permeability, solubility, surface area and fluid movement; is based on work by Grass (1997).
SIMCYP	Is based on global consortium formed by the University of Sheffield and several pharmaceutical companies in 2000 with aim of producing automated platforms for IVIVE in virtual populations. The program incorporates extensive data on demographics, disease states, anatomical, physiological, and genetic and biochemical variables, as well as input of information on in vitro drug metabolism and transport and outputs population distributions of the extent of a metabolic drug-drug interactions.
PK-Sim	'Whole-body' PBPK simulation; description of oral absorption of the compound, systemic distribution of the body, and metabolism and excretion, integrated in one simulation model, introduced by Willmann et al (2003).

3 AIMS OF THE THESIS

The motivating assumption in this thesis is that the best method to construct an in vitro- in vivo correlation (IVIVC) for small-molecule drugs is to use PBPK, in which all relevant physiological processes are accurately described.

Aims of the thesis are:

1. To develop a probabilistic level A IVIVC model to aid in levosimendan modified-release formulation optimisation and to act as a surrogate for BE studies.

2. To use PK modelling of BCS I drugs to demonstrate that very rapid dissolution is not necessary to guarantee their BE.

3. To use PK simulations to explore the dissolution acceptance criteria best suited for BCS I and III biowaivers and to examine the effect of MDR-1 efflux transporter on BE of substrates.

4 MATERIAL AND METHODS

4.1 BAYES IVIVC (I)

4.1.1 IN VIVO DATA

Concentration data from three BA studies for levosimendan- modified-release formulations A–F were used in the IVIVC model using a Bayesian approach. Formulation E was the same as formulation A and, formulation F was the same as C, except that the dose of levosimendan was 1 mg in E and F and 2 mg in A and C. Main characteristics of the three BA studies are shown in Table 3.

Table 3. *Main characteristics of BA studies where levosimendan modified-release formulations A-F were studied.*

BA study	Description	Used for
1	Formulation C studied in ten subjects	Prior data
2	Formulations A, B, C, and D studied in 9 subjects	Formulations A and D for likelihood function All formulations A, B, C, and D for testing internal predictability
3	Formulations E, and F studied in 15-16 subjects	Testing external predictability

4.1.2 IN VITRO DATA

The release rate of all levosimendan modified–release formulations A - F were studied using the United States pharmacopoeia (USP) basket method (rotation speed, 100/min) in sink conditions with phosphate buffer (pH 5.8, 500 mL, 37 °C). A UV spectrophotometre at 210 nm was used to analyse the concentrations of levosimendan in the samples. Each formulation was tested by six parallel dissolution measurements.

The average dissolution data in fractions for each formulation (scaled to intervals of 0-1) were fitted to the following equation to obtain the dissolution rate constants (K_d) for all formulations:

(20) $$Diss(t) = 1 - e^{-K_d \cdot t},$$

where Diss(t) is the cumulative fraction of dissolved drug and t is time. The underlying assumption of this equation was that the residual variability is homoscedastic. The dissolution data were fitted as a separate data pre-processing steps.

4.1.3 IVIVC MODEL WITH BAYESIAN APPROACH

4.1.3.1 Structural model to describe levosimendan concentration-time profile

The dissolution and permeability processes were simultaneously determined using the IVIVC model. To account for the time delay between the drug input rate into the plasma and in vivo dissolution, the lag time (t_{lag}) was included in the model. The in vitro dissolution was described using the dissolution rate constant (K_d) which was calculated separately for all formulations (A, B, C, D, E, and F). In addition, a time scaling factor (a) was used to scale in vitro dissolution rate constant (K_d) to an in vivo absorption rate constant ($K_a = a \cdot K_d$). Therefore, the plasma -concentration profiles were predicted directly using in vitro K_d constants without any additional analysis.
The equation used to describe the levosimendan concentration-time (C(t)) profiles is shown below:

$$(21) \qquad \begin{cases} C(t) = 0, & t \le t_{lag} \\ C(t) = \frac{a \cdot K_d \cdot F \cdot D_{po}}{V_c \cdot (a \cdot K_d - k_{10})} \cdot \left\{ e^{-k_{10} \cdot (t - t_{lag})} - e^{-a \cdot K_d \cdot (t - t_{lag})} \right\}, & t > t_{lag} \end{cases},$$

where a is the time scaling factor between in vitro and in vivo dissolution, K_d is the dissolution rate constant, F is the total BA, D_{po} is the per oral dose of levosimendan, V_c is the volume of the central compartment, k_{10} is the elimination rate constant, and t_{lag} the lag time of drug absorption. The parameter F could not be solved and this unidentifiability was considered by estimating the combined parameter F/V_c as described in the next paragraph. A one-compartmental model was chosen because it adequately described the data in the preliminary analyses. The two-compartmental model does not provide any significant added benefit.

4.1.3.2 Stochastic model

The variability in the IVIVC model was constructed using the Bayesian approach. The Bayesian IVIVC model is presented as a posterior distribution of model parameters which includes both prior information and a likelihood function, that defines the connection between the model parameters and

observed data. The posterior distribution, which is simulated for all the data simultaneously, can be described as follows:

$$(22) \qquad p(\theta, \sigma^2|y, K_d) \propto \left[\prod_i \prod_j \prod_k N\left(logy_{ijk}\middle|logC_{ij}(t_k;\theta), \sigma^2\right)\right] \cdot \left[\prod_l N_{trunc}(\theta_l|\mu_l, \delta_l^2)\right] \cdot \left[\frac{1}{\sigma^2}\right],$$

where θ is parameter vector that contains parameters a, F/V_c, k_{10} and t_{lag} (subject-specific, that is $t_{lag} = (t_{lag,1}, \ldots, t_{lag,9})$); σ^2 is variance of the observed data under the assumed model; y is observed data; K_d is dissolution rate constant; N() is normal distribution; index i is for formulation; index j is for test subject; index k is for time point; index l is for the element of the parameter vector; t is time; $N_{trunc}()$ is truncated normal distribution; μ is mean of normal distribution; δ^2 is variance of normal distribution; and $[1/\sigma^2]$ refers to noninformative prior distribution (Box 1973).

4.1.3.3 IVIVC model structure

Dissolution is the rate-limiting step in the absorption process when constructing the IVIVC model. The role of in vivo dissolution and absorption was evaluated using the MRT approach. The mean absorption time (MAT) was calculated as only 0.1–0.2 h. Together with the high BA (87%) this indicates high intestinal permeability and rapid absorption of levosimendan. The mean dissolution times for formulations A-F were 0.5–1.5 h, which are several times slower than MAT (0.1–0.2 h). This indicates that dissolution is the rate-limiting step in the absorption of levosimendan by modified-release capsules.

4.1.3.4 Simulations

The Metropolis algorithm was used to simulate the posterior distribution. Three parallel simulation runs with different starting points were used, with 50000 simulated parameter vectors per sequence. The last half of each simulation sequence was pooled; thus total number of simulated parameter vectors was 75000. The first half of each simulation sequence is ignored to reduce the effect of the starting point.

4.2 PK SIMULATIONS FOR BCS CLASS 1 & 3 BIOWAIVERS (II & III)

Simulations were conducted to investigate two questions related to this thesis: to demonstrate that very rapid dissolution is not necessary to guarantee BE for BCS class 1 drugs and to explore the dissolution acceptance criteria best suited for BCS class 1 and 3 biowaivers and to examine the effect of MDR-1 efflux transporter on BE of substrates.

4.2.1 PK SIMULATION MODEL

The structure of the PK model (compartmental absorption and transit [CAT] model + systemic two-compartment model) used to simulate BCS class 1 and 3 drug products is shown in Figure 10.

The PK model requires knowledge of the absorption rate constant parameter (K_a), which was calculated for all real drugs extracted from literature using following equation by Linnankoski et al.:

$$(23) \qquad logK_a = 0.623 + 0.154 \cdot logD_{6.0} - 0.007 \cdot PSA,$$

where log $D_{6.0}$ is log D at pH 6.0 and PSA is the polar surface area (Linnankoski 2006).

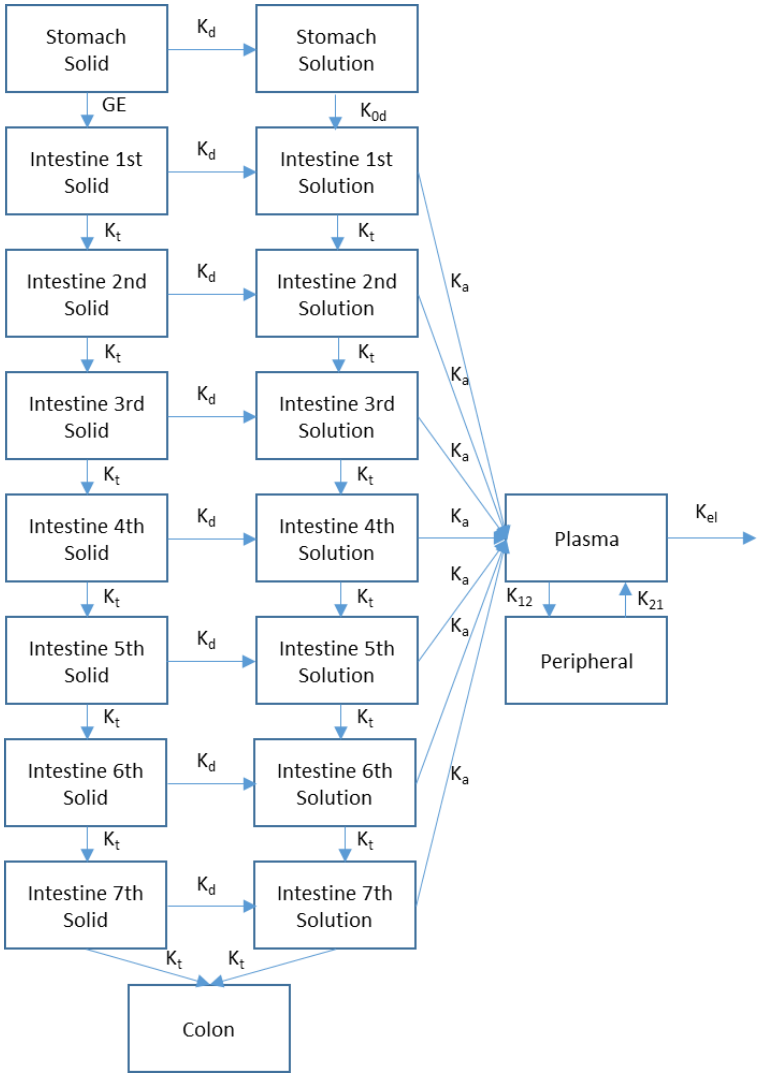


Figure 10 The structure of the PK model (CAT + systemic two-compartment model) and the parameters used in the model: GE is the gastric emptying for solid drugs, K_{0d} is the gastric emptying rate constant for dissolved drugs, K_d is the dissolution rate constant, K_t is the distribution and transit rate constant for solid and dissolved drugs, respectively, K_a is the absorption rate constant, K_{el} is the elimination rate constant, K_{12} is the distribution rate constant into the peripheral compartment and K_{21} is the distribution rate constant into the plasma compartment.

4.2.2 EVALUATION OF RAPID AND VERY RAPID DISSOLUTION CRITERIA FOR BCS CLASS 1 BIOWAIVERS (II)

This thesis aims to investigate whether very rapid dissolution is necessary for biowaived BCS class 1 drugs to guarantee BE. Different dissolution criteria for BCS I biowaivers were evaluated by performing PK simulations for 32 real BCS

I drugs and theoretical values for absorption and elimination. The model used in the simulations is shown in Figure 10: however, the peripheral compartment was omitted. The PK model parameter values used to compare the different dissolution criteria in the simulations are summarised in Table 4 and Table 5. The tablet versus oral solution comparison investigated whether tablet dissolution played any relevant role as a rate-limiting step for overall absorption. The oral solution means that the drug dose is directly administered to "Stomach solution" compartment in Figure 10. In vitro K_d value of 8 approximately correspond to a 85% dissolution within 15 min. In vitro K_d value of 4 approximately corresponds to a 85% dissolution after 30 min. It was unnecessary to investigate the apparent volume of the distribution values because the volume of the distribution did not affect the C_{max} ratios. The investigation explored all the relevant ranges of parameter values for drugs exhibiting one-compartment PK behaviour, since K_a and K_{el} are the only parameters that matter once V_c/F (the volume of distribution/BA) is ruled out.

Table 4. *Dissolution rate constant values that were used in PK simulation model.*

	Very rapid dissolution 85% in 15 min	Rapid dissolution 85% in 30 min	Rapid dissolution and similar dissolution profiles
In vitro K_d (1/h)	8	4	4 vs 5.5
In vivo K_d (1/h)	4	2	2 vs 2.75
Comparison	Tablet vs oral solution		"Slow dissolved" tablet vs "fast dissolved" tablet

Table 5. *Parameter values of real and theoretical (nine different K_a and 12 different K_{el} values, that is, total of 108 combinations) drugs that were used in PK simulation model.*

Parameter	Value
GE (mg/h)	513
dose (mg)	1000
K_{od}	3.47
K_t (1/h)	2.1
K_a range (1/h) for real drugs	0.48-10.9
K_a range (1/h) for theoretical drugs	0.4-12
K_{el} range (1/h) for real drugs	0.0012–0.69
K_{el} range (1/h) for theoretical drugs	0.001–0.9

The ratios of C_{max} for formulation comparison (tablet vs. oral solution or slow-dissolved tablet vs. fast-dissolved tablet) were calculated from the PK

simulations. Real or theoretical drugs with a ratio of C_{max} of >0.9 were considered to have a low risk for bioinequivalence.

4.2.3 EVALUATION OF RAPID AND VERY RAPID DISSOLUTION CRITERIA FOR BCS CLASS 1 AND 3 BIOWAIVERS WITH AND WITHOUT INTERACTION WITH MDR-1 EFFLUX TRANSPORTER (III)

This thesis aimed to investigate which dissolution criteria are best for judging whether a biowaiver can be allowed for BCS class 1 and 3 drugs. This evaluation was performed by simulating PK for 44 real BCS class 1 drugs, 26 real BCS class 3 drugs, and for theoretical drug products. Drugs were classified as BCS class 1 or 3 based on their absorption rate constants being above (BCS 1) or below (BCS 3) 0.8 1/h. The model used in the simulations is shown in Figure 10. When modelling the effect of being a substrate for the MDR-1 efflux protein, it was assumed that absorption was two or five times slower from the ileum (intestinal compartments 4-7) than from the jejunum and duodenum (intestinal compartments 1-3). The simulation scheme is presented in Figure 11. The PK model parameter values used to compare different dissolution criteria with and without interaction with the MDR-1 efflux protein in the simulations are summarised in Table 6 and Table 7. The dissolution rate constant K_d values in Table 6 are slightly different from those in Table 4. Values in Table 6 reflect more accurately exactly 85% being dissolved by 15 or 30 min, and same is reflected also in similar dissolution profiles. The macroparameters displayed in Table 7 were assumed to be uncorrelated with the simulated theoretical drugs. Interestingly, only the ratio of macroparameters A and B is needed and not their specific values because the absolute values of A and B will cancel out once the C_{max} or AUC ratios are calculated. This helps reduce the parameter space required for the simulations. The total number of simulated theoretical drugs was 123670.

The ratios of C_{max} and AUC for formulation comparison (tablet vs. oral solution or slow-dissolved tablet vs fast-dissolved tablet) were calculated from the PK simulations. Real or theoretical drugs with ratios between different dosage forms for C_{max} and AUC >0.9 and <1.1 were considered to have a low risk for bioinequivalence. In addition, the time point of maximum plasma concentration T_{max} was evaluated in the simulations for cases in which the C_{max} ratio was <0.9.

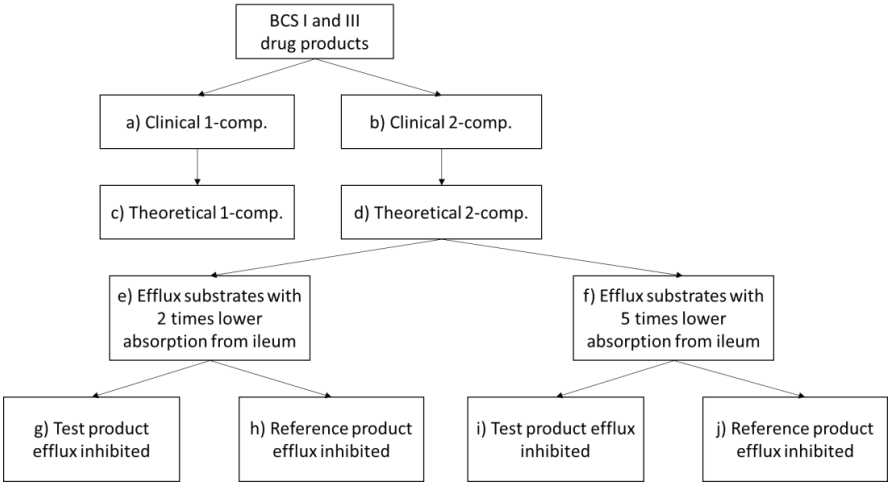


Figure 11 Simulation progress. The starting point for the simulations was clinical one- and two compartment drug products (a and b), which had marketing authorisation. Theoretical simulations have primarily been performed for drug products that follow two- compartment pharmacokinetics. These drug products were simulated without efflux interaction (d), the drug as an efflux substrate but without excipients interacting with efflux transporters (e and f), or efflux was inhibited either in the test (g and i) or reference product (h and j). Figure modified from publication III.

Table 6. *Dissolution rate constant values that were used in the PK simulation model.*

Parameter	Very rapid dissolution 85% in 15 min	Rapid dissolution and similar dissolution profiles
In vitro K_d (1/h)	7.6	3.8 vs. 5.2
In vivo K_d (1/h)	3.8	1.9 vs. 2.6
Comparison	Tablet vs oral solution	"Slow dissolved" tablet vs "fast dissolved" tablet

Table 7. *Parameter values of real and theoretical drugs used in the PK simulation model.*

Parameter	Value
GE (mg/h)	590
dose (mg)	1000
K_{od}	3.47
K_t (1/h)	2.1
One-compartment drugs	
K_a range (1/h):	
real drugs	0.076–5.305
theoretical drugs	0.038–10.611
K_{el} range (1/h):	
real drugs	0.004–0.693
theoretical drugs	0.002–1.386
Two-compartment drugs	
K_a range (1/h):	
real drugs	0.03–7.082
theoretical drugs	0.015–14.164
α range (1/h):	
real drugs	0.642–13.555
theoretical drugs	0.321–27.110
β range (1/h):	
real drugs	0.008–1.560
theoretical drugs	0.004–3.120
A/B range:	
real drugs	0.574–11.396
theoretical drugs	0.287–22.792
K_{21} (1/h)	$\frac{\alpha + \beta \cdot A/B}{1 + A/B}$
K_{el} (1/h)	$\frac{\alpha \cdot \beta}{K_{21}}$
K_{12} (1/h)	$\alpha + \beta - K_{21} - K_{el}$

5 RESULTS

The main results of this thesis are summarised in this chapter. A detailed presentation of these results can be found in Publications I- III.
Publication I provides a successful example of the development of a probabilistic level A IVIVC model for levosimendan.
In Publication II, PK simulations for real 32 BCS I drugs and theoretical values for absorption and elimination rate constants demonstrated that there is no need to tighten the dissolution criterion from rapid to very rapid for BCS class 1 drugs.
In Publication III, the optimal dissolution criteria for BCS class 1 and BCS class 3 biowaivers were proposed, with and without interaction with the MDR-1 efflux transporter by performing large number of PK simulations using the CAT model combined with a 1- or 2-compartment model. It was found that for BCS I drug products that for T_{max}<0.6 h, there is risk for bioinequivalence irrespective of the dissolution criteria, and when 0.6 h<T_{max}<0.9 h, one should use "very rapid" dissolution criterion and if T_{max}>0.9 h, both dissolution criteria "rapid and similar" and "very rapid" are suitable. In addition, using both dissolution criteria, all BCS III drug products were suitable as biowaivers. In addition, when drug products or an excipient is inhibitors of MDR-1 efflux transporter, there is risk of bioinequivalence for almost all BCS III drug products (89%) and for several (9 – 57%) of BCS I drug products. Thus, to avoid the risks of bioinequivalence, excipients with prior use in bioequivalent products should be used.

5.1 DESCRIPTION OF VARIABILITY IN LEVEL A IVIVC MODEL WITH BAYESIAN APPROACH [I]

Dissolution limits the rate of absorption of levosimendan MR formulations. The PK profiles of the different levosimendan MR formulations were described and predicted using a level A IVIVC model with a Bayesian approach to describe variability.
The predictive ability of developed IVIVC model was evaluated comparing predicted marginal posterior distributions $AUC_{0-\infty}$, C_{max} and plasma-concentration-time profiles to observed ones. Internal predictability was evaluated using data from four formulations A, B, C, and D from BA Study 2, in which PK was studied with nine subjects in a cross-over fashion. External predictability was evaluated using data from two formulations, E and F, from BA Study 3, in which PK was studied with 15-16 subjects in a cross-over fashion. The observed and predicted plasma -concentration profiles of the formulations for testing the internal predictability (A, B, C and D) and the external predictability (E and F) are shown in Figure 12.

There were slight differences when comparing the observed concentration-time profiles with the predicted ones for internal formulations A–D. For external formulations, the predicted plasma-concentration profiles were well consistent with the observed curves.
We concluded that the model was predictive both for formulations used in model development and for formulations not used in model development.

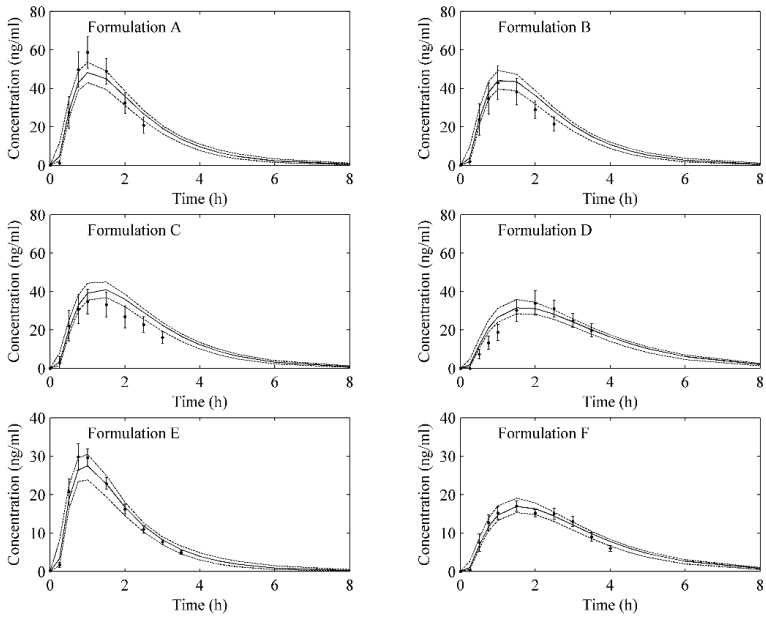


Figure 12 Internal and external predictability of the IVIVC model: observed average values of the plasma concentration-time profile±SEM (dots with error bars), predicted 95% posterior probability interval curves for average levosimendan concentrations in plasma (dashed lower and upper lines) and MAP values (continuous middle line) for modified-release capsulesA – F.

An alternative multi-step approach was also attempted, where %absorbed was first calculated, and then compared with average %dissolved. Nonlinear model was built to relate these two quantities, and this nonlinear model was then used to predict the time-concentration profiles for formulations E and F. Prediction errors of C_{max} were 15% and 70% for formulations E and F respectively, indicating poor performance of this multi-step approach. For this dataset, the one-step approach of directly correlating the K_d values to absorption rate constants was better than the multi-step approach.

5.2 EVALUATION OF DISSOLUTION CRITERIA FOR BCS CLASS 1 DRUGS USING PK SIMULATIONS [II]

5.2.1 DESCRIPTION OF PK SIMULATIONS FOR EXPLORATION OF DISSOLUTION CRITERIA

A CAT model combined with a one-compartment systemic PK model was used in the simulations to explore the dissolution criteria for BCS I drugs to qualify as biowaivers. Because the C_{max} parameter is more sensitive to changes in the dissolution rate than the AUC, it was used in simulations to compare formulations. Tablet and oral solution formulations were simulated, where the dissolution rate constants (K_d) were chosen to represent different dissolution criteria by the WHO, FDA and EMEA (currently EMA). When the C_{max} ratio between the two formulations in simulations was >0.9, the drug was considered to have a low risk of failure in the BE study. A time scale factor of two was introduced to describe different conditions between in vivo and in vitro dissolution.

Simulations were performed for 32 BCS I drugs with different absorption and elimination properties where elimination rates varied from 0.0012 to 0.69 h^{-1} and absorption rate constants varied from 0.48 to 10.9 h^{-1}. In addition, theoretical simulations were performed to further explore the sensitive regions of the BE.

The simulation results are shown in Figure 13. This figure outlines the predicted C_{max} ratios as a functions of absorption and elimination rate constants. Data are shown for 32 real drugs, and the contours represent the predictions for any hypothetical drug with an absorption rate constant in the range 0–12 1/h, and elimination rate constant in the range 0–0.9 1/h. The leftmost subfigure shows the predictions for very rapid dissolution, where a tablet of which 85% dissolution in 15 min is compared to an oral solution, that is, instant dissolution, this comparison represents the maximal possible between-product dissolution rate difference in the case where both products can dissolve 85% in 15 min or less. A risk of bioinequivalence (C_{max} ratio < 0.9) is not present at all within the graph, which supports the current contention that a dissolution-based biowaiver can be applied to very rapidly dissolved BCS class 1 drugs.

The subfigure in the centre shows the results for rapid dissolution, that is, a scenario which a tablet of which 85 % dissolves in 30 min is compared to an oral solution, that is, instant dissolution, this comparison represents the maximal possible between-product dissolution rate difference in the case where both products are able to dissolve 85% in <30 min. The chosen in vitro K_d values of 4 and 5.5 can be considered a worst-case scenario because even though these K_d values produce dissolution profiles that are similar for average profiles, it is possible that if intra-product variability was included the profiles may not be similar (Shah 1998).

A combination of a high elimination rate constant and fast absorption can lead to a risk of bioinequivalence (gray area in the subfigure). This suggests that rapid dissolution alone is not a sufficient for a dissolution-based biowaiver, which is in line with current regulatory requirements.
Finally, the subfigure on the right shows the results for the scenario with rapid dissolution and similar dissolution profiles. It features a slower dissolving product, of which 85% is dissolved within 30 min, and a faster-dissolving product, which still has a similar dissolution profile when compared to the slower-dissolving product. This provided a representative comparison of the products with rapid dissolution and similar dissolution profiles. The subfigure indicates that the risk of bioinequivalence was not present in the relevant parameter ranges investigated in this study. This provides scientific justification for the application of biowaiver to rapidly dissolving drug products with similar dissolution profiles.

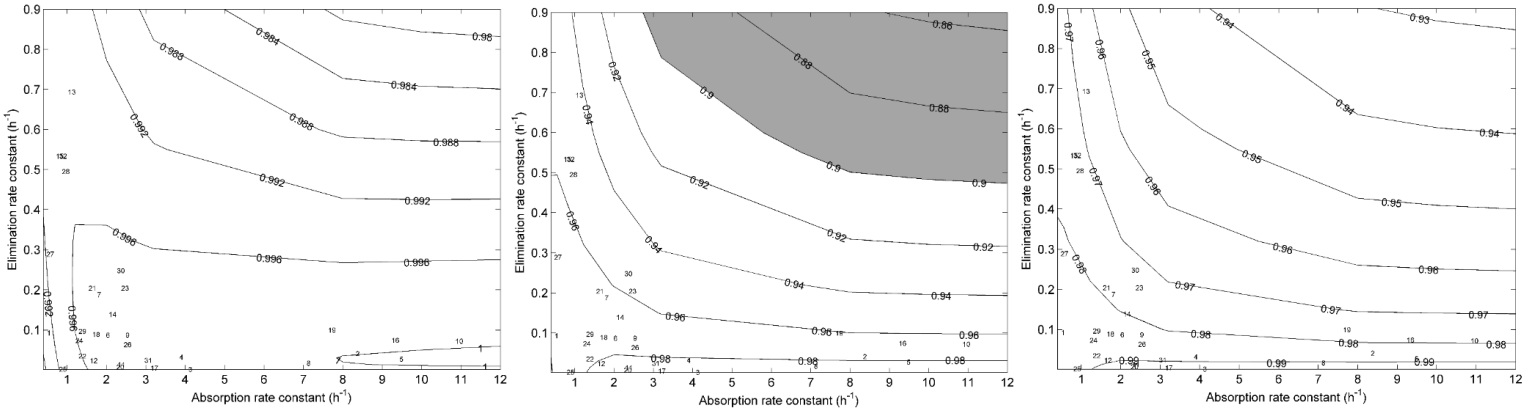


Figure 13 Cmax ratios of BCS I drugs with different dissolution requirements: (left) very rapid dissolution, (middle) rapid dissolution, and (right) rapid dissolution with similar dissolution profiles. In the shaded area of the contour plot Cmax difference is greater than 10%.. Each drug is presented as a number from 1 to 32. 1 Amiloride, 2 Amitriptyline, 3 Chloroquine, 4 Chlorpheniramine, 5 Clomipramine, 6 Cyclophosphamide, 7 Dexamethasone, 8 Diazepam, 9 Diethylcarbamazine, 10 Ethinylestradiol, 11 Ethosuximide, 12 Fluconazole, 13 Isoniazid, 14 Levamisole, 15 Levodopa, 16 Levonorgestrel, 17 Levothyroxine, 18 Nicotinamide, 19 Norethindrone, 20 Phenobarbital, 21 Prednisolone, 22 Proguanil, 23 Propranolol, 24 Pyrazinamide, 25 Pyridoxine, 26 Quinine, 27 Salbutamol, 28 Stavudine, 29 Theophylline, 30 Verapamil, 31 Warfarin, 32 Zidovudine.

5.3 EXPLORATION OF DISSOLUTION CRITERIA FOR BCS CLASS 1 AND 3 BIOWAIVERS WITH AND WITHOUT MDR-1 EFFLUX TRANSPORTER USING PK SIMULATIONS [III]

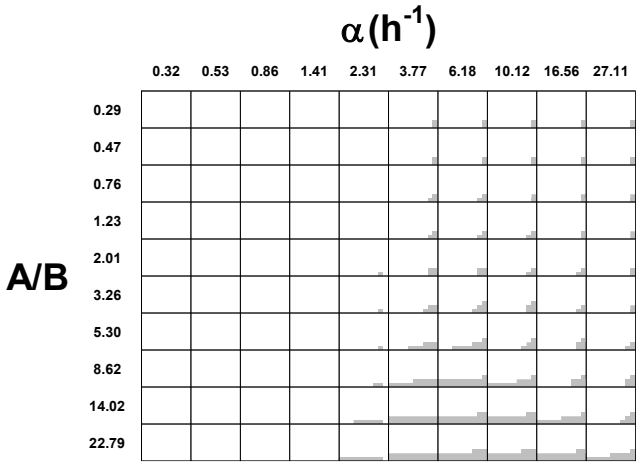
A CAT model combined with a one- or two-compartment systemic PK model was used in the simulations to explore two different dissolution criteria for BCS class 1 and 3 biowaivers with or without interaction with the MDR-1 efflux transporter.

In the simulations test and reference formulations dissolution rate constants (K_d) where chosen to represent two different dissolution criteria: very rapid, or rapid and similar. When simulating the effect of the drug as an MDR-1 substrate, the model was modified such that absorption was two or five times slower from the ileum (last four compartments of the intestine in the CAT model) than from the jejunum and duodenum (the first three compartments of the intestine in the CAT model)

Both the C_{max} and AUC values were simulated for the test and reference products according to two different dissolution criteria with or without interaction with the MDR-1 efflux transporter, for BCS class 1 and 3 drugs. The limit of 0.9 for C_{max} and AUC ratios between the test and reference formulations were considered indicators of low risk of failure in the BE study. Simulations were performed for 44 BCS class 1 and 26 BCS class 3 drugs. In addition, a wide range of theoretical simulations was performed, including 123670 parameter combinations. Simulation results for theoretical two-compartment BCS class 1 and 3 drug products with dissolution criterion "rapid and similar" are shown in Figure 14. The gray colour indicates areas where the C_{max} ratio is <0.9 which can be considered a risk for bioinequivalency. The results shown in Figure 14 indicate that the risk of bioinequivalency is higher for two-compartment drugs that have a pronounced distribution phase (subfigure A) and are rapidly absorbed (subfigure B). Results are presented both for BCS class 1 (K_a > 0.8 1/h) and class 3 (K_a ≤ 0.8 1/h) drugs together in Figure 14. The percentages of theoretical two-compartment BCS class 1 and 3 drug products with a risk of bioinequivalence for both dissolution criteria and with or without interaction with the MDR-1 efflux transporter are shown in Table 8, in the absence of efflux, only BCS class 1 drugs were shown to have a risk of bioinequivalence in some cases, and BCS class 3 drugs were not shown to have a risk of bioinequivalence in any of the cases because the risk of bioinequivalence is associated with rapid absorption, and none of the BCS class 3 drugs, by definition, have rapid absorption. The results comparing the minimum values of the C_{max} ratios with different dissolution criteria for BCS class 1 and 3 drug products, following either one-or two-compartment kinetics for real and theoretical drugs are shown in Table 9.

For cases where the C_{max} ratio was less than 0.9, the time point of the maximum drug concentration (T_{max}) in the plasma was also simulated. For BCS I drug products where T_{max}<0.6 h (corresponding to $K_a \geq 4$ h^{-1} and $\alpha \geq 3.4$ h^{-1}), there is risk for bioinequivalence irrespective of the dissolution criteria used. When T_{max} is between 0.6 h and 0.9 h (corresponding to 2.2 $h^{-1} \leq K_a < 4$ h^{-1} and 1.9 $h^{-1} \leq \alpha < 3.4$ h^{-1}), it is better to use "very rapid" dissolution criterion. If T_{max} for drug product is greater than 0.9 h, then both dissolution criteria "rapid and similar" and "very rapid" are suitable. Based on simulations, it was also clear that all BCS class 3 drug products are suitable biowaivers using both dissolution criteria. When drug product or an excipient is inhibitor of MDR-1 efflux transporter, there is risk of bioinequivalence for almost all BCS class 3 drug products (89%) and for several (9 – 57%) of BCS class 1 drug products. To avoid these risks, excipients with prior use in bioequivalent products must be used.

A



B

A/B=22.79, α=16.56

β (h-1)

Ka (h-1)	0.0040	0.0084	0.0176	0.0368	0.0772	0.1617	0.3389	0.7103	1.4887	3.1200
0.015	0.97	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.97	0.97
0.032	0.97	0.97	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.97
0.069	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.97
0.147	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.97	0.96
0.315	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.96	0.95
0.674	0.98	0.98	0.98	0.98	0.97	0.97	0.96	0.95	0.94	0.94
1.444	0.95	0.95	0.94	0.94	0.94	0.94	0.94	0.93	0.92	0.91
3.091	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.90	0.89	0.89
6.616	0.89	0.88	0.88	0.88	0.88	0.88	0.88	0.87	0.87	0.86
14.164	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.86	0.86	0.85

Figure 14 Simulations for theoretical 2-compartment BCS class 1 and 3 drugs with dissolution criterion ‘‘rapid and similar’’ without efflux interaction. Gray signifies areas where the C_{max} ratio is <0.9 indicating a risk of failing a BE study. Table (A) represent all 8800 simulations and each small square contains the same parameter ranges for k_a and β as presented in table (B) for parameters A/B = 22.79 and α = 16.56.

Table 8. *The percentages of theoretical two-compartment BCS class 1 and 3 drug products having C_{max} or AUC (in parenthesis) ratio < 0.9 or >1.1 with different dissolution criteria. Effl. subst. 2x or 5x lower represents the situation, when the drug is a substrate for efflux and thus drug has lower absorption from ileum than duodenum and jejunum. Efflux inhibited refers to the case where the excipient has inhibited the efflux protein.*

	Test	Reference	Very rapid percent of ratios < 0.9 or >1.11	Rapid and similar percent of ratios < 0.9 or >1.11
BCS 1	Theoretical 2-comp	Theoretical 2-comp	6	11
	Effl. subst. 2x lower	Effl. subst. 2x lower	5	11
	Effl. subst. 5x lower	Effl. subst. 5x lower	6	14
	Efflux inhibited	Effl. subst. 2x lower	6 (0)	9 (0)
	Efflux inhibited	Effl. subst. 5x lower	11 (0)	18 (25)
	Effl. subst. 2x lower	Efflux inhibited	6 (0)	32 (0)
	Effl. subst. 5x lower	Efflux inhibited	20 (25)	57 (25)
BCS 3	Theoretical 2-comp	Theoretical 2-comp	0	0
	Effl. subst. 2x lower	Effl. subst. 2x lower	0	0
	Effl. subst. 5x lower	Effl. subst. 5x lower	0	0
	Efflux inhibited	Effl. subst. 2x lower	89 (100)	97 (100)
	Efflux inhibited	Effl. subst. 5x lower	96 (100)	97 (100)
	Effl. subst. 2x lower	Efflux inhibited	94 (100)	100 (100)
	Effl. subst. 5x lower	Efflux inhibited	99 (100)	100 (100)

Table 9. *Comparison of minimum values of C_{max} ratios with different dissolution criteria of BCS class 1 and 3 drug products, following either one-or two compartment kinetics.*

	Test	Very rapid C_{max} ratio min values	Rapid and similar C_{max} ratio min values
BCS 1	Real 1-comp.	0.99	0.95
	Theoretical 1-comp.	0.96	0.91
	Real 2-comp	0.98	0.94
	Theoretical 2-comp.	0.75	0.85
BCS 3	Real 1-comp.	0.98	0.97
	Theoretical 1-comp.	0.98	0.96
	Real 2-comp	0.98	0.97
	Theoretical 2-comp.	0.98	0.94

6 DISCUSSION

The overall goal of this thesis is to investigate the best method to construct an IVIVC/IVIVR in which all relevant physiological phenomena and variability are accurately described. A crucial step in constructing IVIVC/IVIVR is to determine the in vitro method that best describes the in vivo conditions by optimising the in vitro testing conditions. The probabilistic level A IVIVC model in Publication I was successfully established using the Bayesian approach. Successfully constructed IVIVC is a valuable tool for formulation optimisation during drug development. As several parameters affect the in vivo absorption process, it is important to use simulations to determine the areas in the multidimensional parameter space which may contain a risk of bioinequivalence for BCS class 1 and 3 drugs. In Publications II and III, a significant number of simulations were performed to investigate the role of dissolution criteria for IR small-molecule drug products to qualify as biowaivers. BCS class-based biowaivers can greatly aid generic drug development by decreasing the need for BE studies.

Owing to the long time period of this thesis, it is structured such that the literature review contains state-of-the-art until the start of the thesis studies (2005). Research conducted in the field after the start of this thesis (2005) is presented in this chapter, and own publications are compared with more recent work in this field of science. Important papers for the prediction of PK of small-molecule drugs using modelling methods for establishing relationship between dissolution data and in vivo absorption is shown in Table 10.

Table 10. *Important papers for the prediction of PK of small-molecule drugs using modelling methods for establishing relationship between dissolution data and in vivo absorption after 2005*

Year	Brief description and importance	Full reference
2006 (own)	First application of probabilistic level A IVIVC model using Bayesian approach for modified-release formulation series of levosimendan.	Kortejärvi H, Malkki J, Marvola M, Urtti A, Yliperttula M, Pajunen P. Level A in vitro-in vivo correlation (IVIVC) model with Bayesian approach to formulation series. J Pharm Sci 95, 1595-605 (2006).
2008	Statistically well constructed comparison of convolution versus deconvolution for establishing IVIVC.	Gaynor C, Dunne A, Davis J. A comparison of the prediction accuracy of two IVIVC modelling techniques. J Pharm Sci 97, 3422-32 (2008).
2009	Very rapid dissolution criterion were shown to be too strict criterion on BE using Gastroplus for four real drugs.	Kovacević I, Parojcić J, Homsek I, Tubić-Grozdanis M, Langguth P. Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. Mol Pharm 6, 40-7. (2009).
2010 (own)	The use of PK simulations for 32 real and theoretical drugs to show that very rapid dissolution criterion for BCS class 1 drugs is too strict.	Kortejärvi H, Shawahna R, Koski A, Malkki J, Ojala K, Yliperttula M. Very rapid dissolution is not needed to guarantee bioequivalence for biopharmaceutics classification system (BCS) I drugs. J Pharm Sci 99, 621-5 (2010).

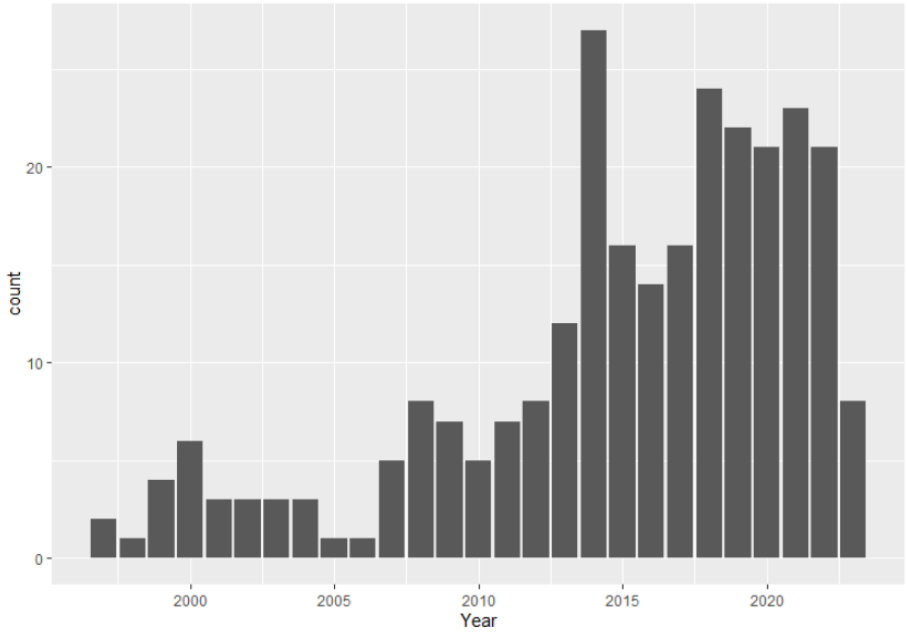
Year	Brief description and importance	Full reference
2010	Real BCS class 1 and 3 drugs were used to test suitability of gastrointestinal simulation software (Gastroplus) for predicting drug absorption. In addition, it was shown using silico BE studies that current dissolution criteria are conservative and it is feasible to allow biowaivers for IR dosage forms of BCS class 3 drug products.	Tsume Y, Amidon GL. The biowaiver extension for BCS class III drugs: the effect of dissolution rate on the bioequivalence of BCS class III immediate-release drugs predicted by computer simulation. Mol Pharm 7, 1235-43 (2010).
2012	Suitability of in silico prediction of BCS class 2 weak acid drugs for biowaivers were evaluated using in silico software Gastroplus. It was noticed that the oral absorption of these drugs depend highly on the intestinal pH and buffer strength. These factors must be taken into account for a BE test.	Tsume Y, Langguth P, Garcia-Arieta A, Amidon GL. In silico prediction of drug dissolution and absorption with variation in intestinal pH for BCS class II weak acid drugs: ibuprofen and ketoprofen. Biopharm Drug Dispos 33, 366-77 (2012).
2012	A nonlinear mixed-effects model was used to describe the in vitro drug release. This new modelling approach in prediction of in vivo behavior from standard in vitro experiments can be used to support formulation development and quality control.	Bergstrand M, Söderlind E, Eriksson UG, Weitschies W, Karlsson MO. A semi-mechanistic modeling strategy to link in vitro and in vivo drug release for modified release formulations. Pharm Res 29, 695-706 (2012).
2014 (own)	Extensive PK simulation study to evaluate the dissolution criteria for BCS class 1 and 3 biowaivers. Also the role of efflux transporter were explored. Clear criteria for accepting BCS class 1 and 3 drugs were suggested.	Kortejärvi H, Malkki J, Shawahna R, Scherrmann JM, Urtti A, Yliperttula M. Pharmacokinetic simulations to explore dissolution criteria of BCS I and III biowaivers with and without MDR-1 efflux transporter. Eur J Pharm Sci 61, 18-26 (2014).
2014	Thorough evaluation of using different methods in predicting gastrointestinal (GI) transfer. Methods evaluated included in vivo approach, in vitro approach TIM, and in silico approach SIMCYP. First direct assessment of impact of GI transfer of solutions on intraluminal drug concentrations in humans. This data can be used to validate and optimise in vitro and in silico simulation tools.	Hens B, Brouwers J, Anneveld B, Corsetti M, Symillides M, Vertzoni M, Reppas C, Turner DB, Augustijns P. Gastrointestinal transfer: in vivo evaluation and implementation in in vitro and in silico predictive tools. Eur J Pharm Sci 63, 233-42 (2014).

Year	Brief description and importance	Full reference
2016	Thorough PBPK simulation study to assess the impact of excipients on absorption of different BCS class 1, 2, and 3 IR drug products. PBPK modelling was shown to be a good tool to evaluate different biowaiver requirements.	Chow EC, Talattof A, Tsakalozou E, Fan J, Zhao L, Zhang X. Using Physiologically Based Pharmacokinetic (PBPK) Modeling to Evaluate the Impact of Pharmaceutical Excipients on Oral Drug Absorption: Sensitivity Analyses. AAPS J 18, 1500-1511 (2016).
2016	In silico absorption modeling tool Gastroplus was used to create dissolution safe space for BE, that is, if dissolution time profiles of drug product lie within this area, they can be considered bioequivalent. The good predictive ability of the model was shown using external in vivo clinical trial data.	Pepin XJ, Flanagan TR, Holt DJ, Eidelman A, Treacy D, Rowlings CE. Justification of Drug Product Dissolution Rate and Drug Substance Particle Size Specifications Based on Absorption PBPK Modeling for Lesinurad Immediate Release Tablets. Mol Pharm 13, 3256-69 (2016).
2020	FDA draft guidance gives instructions on how to use PBPK for biopharmaceutical applications such as oral drug product development, manufacturing changes, and controls.	FDA. The Use of Physiologically Based Pharmacokinetic Analyses — Biopharmaceutics Applications for Oral Drug Product Development, Manufacturing Changes, and Controls. Guidance for Industry. DRAFT GUIDANCE (2020).
2020	Summary of IMI OrBito project, where oral biopharmaceutical tools were developed. Project was joined by academia, regulatory agency, pharmaceutical companies, technology companies, and non-profit research organisation. During six years of project, novel and existing biopharmaceutics tools were extensively validated using historical datasets provided by industry partners and laboratory ring studies.	Abrahamsson B, McAllister M, Augustijns P, Zane P, Butler J, Holm R, Langguth P, Lindahl A, Müllertz A, Pepin X, Rostami-Hodjegan A, Sjögren E, Berntsson M, Lennernäs H. Six years of progress in the oral biopharmaceutics area - A summary from the IMI OrBiTo project. Eur J Pharm Biopharm 152, 236-247 (2020).

Year	Brief description and importance	Full reference
2021	Well designed and constructed Bayesian population PBPK absorption model were applied for bupropion hydrochloride oral dosage forms. Both inter- and intra-individual variability was quantified in the model. Sensitivity analysis was used to identify influential parameters. The model was able to accurately characterise in vitro dissolution and in vivo exposure data.	Hsieh NH, Bois FY, Tsakalozou E, Ni Z, Yoon M, Sun W, Klein M, Reisfeld B, Chiu WA. A Bayesian population physiologically based pharmacokinetic absorption modeling approach to support generic drug development: application to bupropion hydrochloride oral dosage forms. J Pharmacokinet Pharmacodyn 48, 893-908 (2021).
2021	Several representative real-life case studies used to illustrate the concept of safe BE space for IR drug products using PBBM.	Heimbach T, Kesisoglou F, Novakovic J, Tistaert C, Mueller-Zsigmondy M, Kollipara S, Ahmed T, Mitra A, Suarez-Sharp S. Establishing the Bioequivalence Safe Space for Immediate-Release Oral Dosage Forms using Physiologically Based Biopharmaceutics Modeling (PBBM): Case Studies. J Pharm Sci 110, 3896-3906 (2021).
2021	Detailed review on how PBPK modelling and simulation methods can be used to develop an IVIVC and to suggest biowaivers for BCS class 3 drugs.	Wu F, Cristofoletti R, Zhao L, Rostami-Hodjegan A. Scientific considerations to move towards biowaiver for biopharmaceutical classification system class III drugs: How modeling and simulation can help. Biopharm Drug Dispos 42, 118-127 (2021).
2023	Thorough review to showcase the current state and challenges for use of PBBM in oral drug development.	Wu D, Li M. Current State and Challenges of Physiologically Based Biopharmaceutics Modeling (PBBM) in Oral Drug Product Development. Pharm Res 40, 321-336 (2023).

6.1 ESTABLISHING IVIVC FOR MR FORMULATIONS WITH VARIABILITY

The number of publications containing the keywords dissolution, IVIVC, or IVIVR is shown in Graphic 2. The number of publications **per year** has increased over the last 25 years.



Graphic 2 Number of publications per year containing key words “dissolution (ivivc OR ivivr) modelling” until 25.4.2023 (Pubmed search)

The Publication I was a probabilistic level A IVIVC model in which the Bayesian approach was used with good predictive capacity. Previous studies have included variability in constructing the IVIVC using mixed-effects modelling (Mauger 1997, Bigora 1997). The Bayesian approach allows for a combination data from different studies and sources. This makes it possible to combine observed data and prior knowledge to obtain results as probability distributions. In Publication I, the in vivo data used to construct and evaluate the internal and external predictability of the IVIVC model using the Bayesian approach consisted of three separate clinical studies. Prior data were obtained using the formulation from Study 1. Two of the four formulations in Study 2 were used as observed data. The remaining two of the four formulations in Study 2 were used to test the internal predictability and two formulations in Study 3 were used to test the external predictability of the model. The goal was

to thoroughly test the predictive ability of the IVIVC model. However, one could argue that even further confidence could have been gained by including more clinical data as observed data, for example, include Study 1 and entire Study 2 as observed data. In addition, it would have been possible to generate prior distribution using earlier knowledge from different compounds or in vitro data combined with in silico tools. This knowledge can be used to create initial estimates of the time scaling factor between in vitro dissolution and in vivo absorption, absorption rate, volume of distribution and clearance (Linnankoski 2006, Poulin 2002, Rowland 2022). An additional topic would have been the simultaneous fitting of the time-dissolution profiles and drug time-concentration profiles. The direct convolution method used in Publication I has been used previously (Buchwald 2003), however it is more common to construct an IVIVC using the deconvolution method, that is, linking the in vitro dissolution profile with the in vivo absorption profile. Direct convolution is a one-step approach in which estimates of drug concentrations in the plasma can directly obtained. However, it may sometimes be more beneficial to first link in vitro dissolved to in vivo absorbed to visualise the possible nonlinearities in correlation, and to aid in dissolution method development toward being more biopredictive.

The development of a mechanistic modelling approach PBPM (physiologically based biopharmaceutics modelling, which is basically the same as PBPK) together with an increasing amount of detailed data on different biopharmaceutical and PK processes provides an interesting method for the construction of IVIVC (Anand 2022). The FDA released draft guideline on the use of this method for oral small-molecule drug development, manufacturing changes, and controls (FDA draft guidance 2020). When linking in vitro dissolution data with in vivo absorption data in the modelling context, it is crucial to realise and describe the differences between in vitro and in vivo conditions. The intestine is not a single compartment where dissolution occurs under constant conditions such as pH and the amount of bile salts (Lindahl 1997, Perez de la Cruz 2006, Clarysse 2009). Addionally, the amount of fluid in the intestine varies as well (Schiller 2005, Mudie 2014).

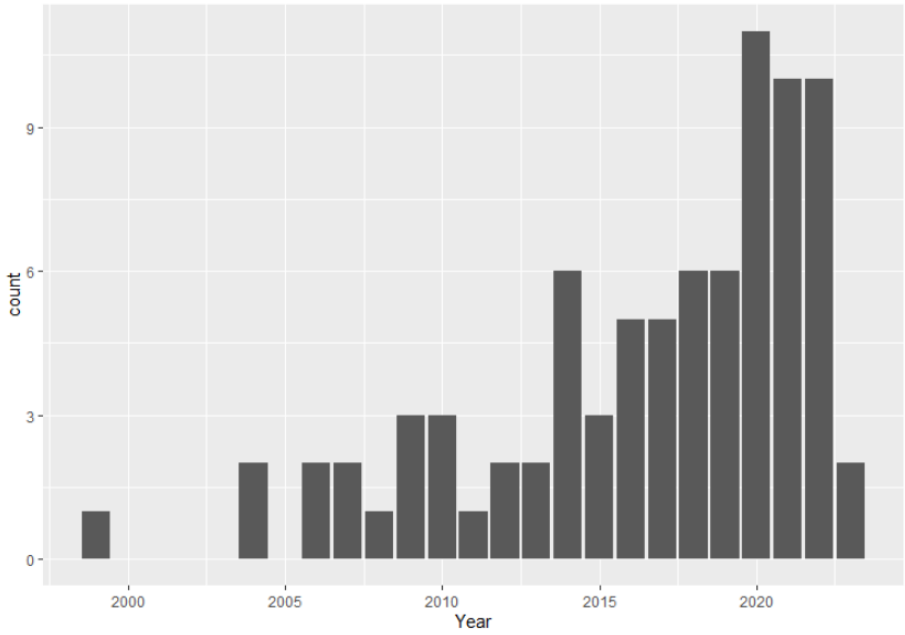
There are variations in the abundance of metabolic enzyme and transporter expression in different segments of GI tract, which causes variance in enteric metabolism and intestinal permeability along the intestine (Berggren 2007, Li 2020, Olivares-Morales 2015; Pade 2017). PBBM is a mechanistic tool used to separately characterise the roles and magnitudes of the aforementioned processes involved in in vivo absorption. Recently, several reviews have been published on the use of PBBM (Wu 2021 ,Anand 2022, Wu D 2023b, Han 2022). Biopredictive dissolution is crucial when establishing an in vitro- in vivo link using PBBM. Several methods to incorporate dissolution data into PBBM exist, as reviewed by Jamei et al. (2020). However, for MR drug products, the direct input of dissolution data and Weibull functions fitted to dissolution data are currently the main options used for creating an IVIVC (Babiskin 2015, Jaiswal 2021, Jereb 2019, Ni 2017, Wu X 2022). Neither of

these methods provide mechanistically accurate descriptions of in vivo dissolution; therefore there is still a lack of mechanistic models for the dissolution of MR products.

A significant aspect to consider when constructing correlation or relationship between in vitro and in vivo data is the description of inter- and intrasubject variability. Especially, it is known that physiological entities such as pH in intestinal tract, osmolarity, bile salts and gastric emptying are variable (Butler 2019, Hens 2017, Parrott 2021, Rabbie 2015, Riethorst 2016). However, although intersubject variability has been characterised for variability in the GI tract, intrasubject variability is more difficult to describe. To accurately describe the extent of intra- and intersubject variability, it is necessary to measure these parameters directly from the GI tract of several subjects and on more than one occasion per individual subject. This in vivo characterisation approach has been used while measuring PK profile of ibuprofen in both the intestine and plasma (Bermejo 2020). However, this approach is invasive because a tube is inserted in GI tract to enable fluid measurements. Bayesian population modelling of the semi-physiological PK absorption model for buprion hydrochloride oral dosage forms was used to characterize intra- and interindividual variability when linking in vitro dissolution and in vivo absorption data (Hsieh 2021). This type of stochastic modelling can capture variability well and can be used to perform virtual BE trials to discover bioequivalent dissolution profiles. Although this type of stochastic modelling enables informative predictions as probability distributions, the amount of data needed to create these models may be a hindrance. To capture both intra- and intersubject and formulation-dependent variability, one typically requires in vivo data from several formulations and preferably same type of formulation should be administered more than once to the same subject. In addition, to accurately describe the differences in GI conditions caused by demographics such as age or disease-related factors the clinical data used to construct the model would need to consist of a wide enough range of these factors. In PBBM models it is possible to evaluate the effect of these factors using mechanistical models and parameters. The Bayesian approach is often more difficult to apply for fully mechanistic models owing to computational issues and lack of observed data for accurately estimating a large set of parameters: However, despite these complexities, this method can still be useful in formulation development because of its ability to predict variability by combining variability in observed data and prior knowledge. This was also demonstrated in Publication I, where the Bayesian approach was applied to the IVIVC for the formulation series.

6.2 EXPLORING DISSOLUTION CRITERIA FOR BCS CLASS 1 AND 3 BIOWAIVERS USING PK SIMULATION MODELS

The number of publications containing the keywords dissolution, biowaiver and modelling is depicted in Graphic 3. The number of publications **per year** increased over the last 25 years.



Graphic 3 Number of publications per year containing key words “dissolution biowaiver modelling” until 25.4.2023 (Pubmed search)

6.2.1 SIMULATIONS FOR BCS CLASS 1

The eligibility of BCS class 1 drugs for biowaivers has been evaluated in Publications II and III. It was found that the eligibility for the biowaiver and the suggested dissolution criterion are dependent on the time to reach the maximum concentration (T_{max}) of the drug product.

PK simulations using a CAT model combined with systemic one-compartment model were used to evaluate the need for very rapid dissolution criterion (85% in 15 min) for BCS class 1 biowaivers for 32 real drugs and theoretical values of absorption and elimination rate constants in Publication II. The very rapid dissolution criterion for rapidly dissolving drug products was also found to be conservative when studied using PK simulations for three model drugs with slight differences in the model structures (Kaus 1999). In addition, the need for a very rapid dissolution criterion for four real BCS class 1 drugs was

explored using PBPK modelling approach with Gastroplus simulation software (Kovacevic 2009). Based on these three separate analyses it can concluded that rapid dissolution (85% in 30 min) and similar dissolution profiles should be sufficient for biowaiver justification. In Publication III, the exploration of suitable dissolution criteria was extended by performing simulations with CAT + systemic one- or two-compartment models for BCS class 1 compounds, where their role as substrate for MDR-1 efflux transport was also investigated. It was possible to determine suitable dissolution criteria by categorising the compounds based on differences in T_{max}. A comparison of the biowaiver acceptance criteria (FDA, EMA and WHO) for dissolution, with suggestions based on the simulations, is shown in Table 11. By finding the best and not too stringent dissolution criterion for each compound, biowaivers can be granted using the most optimal method, thus decreasing the need for in vivo studies when it is not scientifically justified. It was also observed that if drugs or excipients of drug product inhibit MDR-1 efflux transporters, there is a risk of bioinequivalence in several BCS class 1 drugs. Although these results concern only MDR-1 inhibitors, the general implication is that excipients which inhibit any relevant transporter in the body can increase the risk of bioinequivalence.

Table 11. *Comparison of the acceptance criteria for dissolution and excipient–efflux interaction of BCS-based biowaivers (class 1) set by the FDA (2000), EMA (2010) and WHO (2006) and suggestions based on simulations.*

	BCS class 1	
	FDA, EMA and WHO	Based on simulations
Dissolution	Very rapid dissolution/Rapid dissolution and similar dissolution profiles at pH 1.2, 4.5 and 6.8	T_{max} < 0.6 h: No biowaiver candidate T_{max} 0.6–0.9 h: Very rapid dissolution T_{max} > 0.9 h: Very rapid dissolution or rapid dissolution and similar dissolution profiles
Criteria to exclude excipient-efflux interaction	Well-known excipients and usual amounts, which is consistent with the intended function in solid immediate release dosage forms. Excipients have been used earlier with API under consideration. FDA in addition: Linear PK, No Pgp-efflux substrate	Well-known excipients and usual amounts, which is consistent with the intended function in solid immediate release dosage forms. Excipients have been used earlier with API under consideration

6.2.2 SIMULATIONS FOR BCS CLASS 3

The eligibility of BCS class 3 drugs for use in biowaivers was investigated using the same simulation model as that for BCS class 1 compounds in Publication III. A comparison of the biowaiver acceptance criteria (EMA and WHO) for dissolution, with suggestions based on the simulations, is presented in Table 12. Using both studied dissolution criteria, it was shown that BCS class 3 drugs are suitable for biowaivers. As shown in Table 8,none of the BCS class 3 drugs were at a risk of bioinequivalence. As the absorption of BCS class 3 drugs is slower, dissolution is less likely to be a bottleneck for the overall absorption processes of these drugs. The use of a less stringent dissolution criterion may increase the number of BCS class 3 drugs accepted as biowaivers, thereby reducing the need for in vivo testing. However, when a drug or excipient of a drug product inhibits the MDR-1 efflux transporter, there is a risk of bioinequivalence for almost all BCS class 1 drugs. This high risk of bioinequivalence is likely present when other transporters are inhibited. Importantly, the criteria for BCS class 3 biowaivers can be justified scientifically considering all relevant processes.

Table 12. *Comparison of the acceptance criteria for dissolution and excipient efflux interaction of BCS-based biowaivers (class 3) set by the EMA (2010) and WHO (2006) and suggestions based on simulations.*

	BCS class 3	
	EMA and WHO	Based on simulations
Dissolution	Very rapid dissolution at pH 1.2, 4.5 and 6.8	Very rapid dissolution/rapid dissolution and similar dissolution profiles at pH 1.2, 4.5 and 6.8
Criteria to exclude excipient-efflux interaction	Qualitatively and quantitatively very similar composition	Well-known excipients and usual amounts, which is consistent with the intended function in solid immediate release dosage forms. Excipients have been used previously with API considered

6.2.3 SIMULATIONS FOR ALL BCS CLASSES

The use of mechanistic approaches such as PBBM to simulate the role of dissolution criteria for biowaivers enables a more accurate description of different processes in in vivo drug absorption (Wu et al 2021). By separately describing the major factors such as shear stress, buffer capacity, and fluid volume within the PBBM, one can describe the drug release variability at the site of absorption. In addition, one can separately account for drug-specific parameters, for example solubility, bile:micelle partition coefficient, formulation-specific parameters such as particle size in these mechanistic

models and GI variability, for example intraluminal pH. These parameters combined with other important knowledge, such as regional differences in the abundances of metabolic enzymes and transporters along the intestinal tract, effect of food, fluid distribution along the intestine, and gastric emptying are processes and parameters that can be individually investigated using PBBM. In addition, knowledge of the actual inter- and intrasubject variability in different physiological parameters can be incorporated into these mechanistic models.

PBBM models have been successfully used to create a safe BE space for new bioequivalent formulations and to set clinically relevant in vitro specifications to ensure drug product quality (Wu 2022). Several case studies for drugs from different BCS classes has been presented for applications such as how to set in vitro dissolution/particle size distribution specifications, widening dissolution specification to supersede f2 tests, or application toward a scale-up and post-approval changes biowaiver. This type of mechanistic modelling, combined with relevant biopredictive dissolution data, can aid in avoiding unnecessary BE studies in healthy volunteers. Therefore, it is necessary that the acceptance of simulation tools such as PBBM be further endorsed in both new and generic drug development.

6.3 FUTURE PROSPECTS

The use of in vitro dissolution data to predict in vivo absorption through PK modelling has seen significant advances over the past decades. The rise of mechanistic PBPK modelling (or equivalently PBBM), together with emerging knowledge and detailed data on different processes and phenomena related to GI absorption, such as abundance, location, and intersubject variability of metabolic enzymes and transporters in the intestine has led to the possibility of a more accurate and realistic prediction of in vivo behavior based on in vitro dissolution data. This is particularly true for IR small-molecule drug products. There is room for improvement in the mechanistic description of the in vivo release/dissolution process of MR/ER oral small-molecule drug products. There will be an increasing number of studies focusing on a detailed physiological description of how different components and excipients of formulations affect the release, dissolution, metabolic, and permeation processes in the near future. In addition, a clear future focus will be on improving the description of intra- and intersubject variability in PBPK models. Recent advances in artificial intelligence and machine learning will also impact how in vitro- in vivo relationships will be constructed in the future (Terranova 2023). It will automate several parts of the process and hopefully enable the optimal use of all available data. However, scientific progress in predicting in vivo absorption based in vitro dissolution data will still depend on new ideas from experienced biopharmaceutical scientists, PK experts, pharmacometricians, and physicians.

7 CONCLUSIONS

In this study, a level A IVIVC model with a Bayesian approach was developed for modified-release formulation series of levosimendan. The model quantifies the relationship between in vitro dissolution data and in vivo concentrations, and can be used to waive BE studies both in new oral drug formulation development and in generic drug development.

In this thesis, PK simulations in relevant multidimensional parameter spaces were used to set the appropriate dissolution criteria for both BCS class 1 and 3 IR small-molecule drugs. The effect of the MDR-1 efflux transporter on the BE of the substrates was also evaluated. Relevant updates to the current regulatory biowaiver criteria for BCS class 1 and 3 IR small molecule drugs have been proposed.

This thesis contributes to the overall scientific discussion of quantifying the relationship between in vitro dissolution and in vivo concentration data. Finally, it was concluded that the best and most accurate method to create an in vitro- in vivo link is through the use of in vivo relevant physiological and drug-specific parameters.

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