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Modelling and simulation approaches for waiving *in vivo* pharmacokinetic formulation studies

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ACADEMIC DISSERTATION

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Abstract

The bioavailability and bioequivalency of oral drug depends on gastrointestinal tract physiology and drug-related physicochemical and pharmacokinetic factors. In general, bioavailability of a new drug substance or new formulation is studied *in vivo* with healthy volunteers. *In vivo* bioequivalency studies are needed for generic drug products or if a formulation is significantly altered during clinical trials. In certain cases, *in vitro* dissolution studies can be used as a surrogate for *in vivo* bioavailability or bioequivalency studies, referred to as a "biowaiver". These biowaivers are based either on *in vitro-in vivo* correlation (IVIVC) or the biopharmaceutical classification system (BCS). For drugs with dissolution rate-controlled absorption and level A IVIVC, a direct relationship between *in vivo* drug input and *in vitro* dissolution may be found. A BCS biowaiver can be utilised for BCS I drugs that have complete absorption due to high solubility and high permeability.

In this thesis, *in vitro* dissolution methods and computer simulation models were developed to predict relative bioavailability and bioequivalency and to probe properties of drugs suitable for biowaivers. A level A IVIVC model with a stochastic approach was developed for a modified-release formulation series of levosimendan. Firstly, the criteria for selection of a dissolution method for the level A IVIVC model were evaluated. Secondly, a stochastic Bayesian approach was integrated with the level A IVIVC model in order to get a predictions of concentration-time profiles of different formulations.

BCS biowaiver studies included literature data evaluation of immediate release formulations of ranitidine, a BCS III drug with high solubility and low permeability. Ranitidine was evaluated as a potential BCS biowaiver candidate. Generalised rules to estimate the risk of bioinequivalency and to suggest new potential biowaivers were evaluated by theoretical pharmacokinetic simulations. Gastrointestinal tract physiology, formulation type and drug solubility, dissolution, absorption and elimination rates were taken into account in the pharmacokinetic simulation model.

A dissolution method using pH 5.8 and a rotation speed of 100rpm/min provided acceptable discrimination between formulations based on the level B IVIVC and comparisons of pharmacokinetic parameters (MRT and T_{max}) to the dissolution profiles for levosimendan. The level A IVIVC model with Bayesian approach has good external predictability for the formulation series, although an averaged IVIVC model with the same data failed. Subject-specific *in vivo* data was utilised and predictions were obtained as probability distributions. The BCS III drug ranitidine was suggested as a biowaiver candidate based on data from the literature. Generally, the simulations suggest that BCS III drugs are better biowaiver candidates than some BCS I drugs because they have a lower risk of bioinequivalence and they are less sensitive to differences in gastric emptying rates and formulation types. BCS I drugs are currently accepted for biowaivers, although a short half-life of elimination and rapid rate of absorption cause a high risk of bioinequivalency.

Pharmacokinetic models were constructed and tested to predict in vitro-in vivo correlations, relative bioavailability, risk of bioinequivalency and potential for biowaivers. These models are useful new tools in formulation development and regulatory applications.

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Hanna Kortejärvi

Definition of terms

Bioavailability	Bioavailability is defined as the relative fraction of a drug dose that enters the systemic circulation
Bioequivalence	Bioequivalence of a drug product is achieved if its extent and rate of absorption are not statistically significantly different from those of the standard when administered at the same molar dose
Biowaiver	The regulatory acceptance of *in vitro* testing as a reliable surrogate for an *in vivo* bioequivalence study is commonly referred to as biowaiver
Level A IVIVC	A predictive mathematical model for the relationship between the entire *in vitro* dissolution/release time course and the entire *in vivo* response time course, e.g., the time course of drug concentration in plasma or amount of drug absorbed
Input profile	*In vivo* dissolution or *in vivo* absorption (includes permeability and dissolution phases) of the drug from a particular dosage form
IVIVC	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, e.g., drug concentration in plasma or amount of drug absorbed

List of original publications

I Kortejärvi H, Mikkola J, Bäckman M, Antila S, and Marvola M. 2002. Development of level A, B and C *in vitro-in vivo* correlations for modified-release levosimendan capsules. *Int. J. Pharm*. 241:87-95

II Kortejärvi H., Malkki J., Marvola M., Urtti A., Yliperttula M. and Pajunen P. 2006. Level A *in vitro-in vivo* correlation (IVIVC) model with Bayesian approach to formulation series. *J. Pharm. Sci* 95(7): 1595-1605

III Kortejärvi H., Yliperttula M., Dresmann J.B., Junginger H.E., Midha K.K., Shah V.P. and Barends D.M. 2005. Biowaiver Monographs for Immediate Release Solid Oral Dosage Forms: Ranitidine Hydrochloride. *J. Pharm. Sci* 94(8): 1617-1625.

IV Kortejärvi H., Urtti A., and Yliperttula M. 2007 Pharmacokinetic simulation of biowaiver criteria: the effects of gastric emptying, dissolution and absorption and elimination rates *Eur. J. Pharm. Sci* 30(2): 155-166.

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1 Introduction

After oral administration, drugs are exposed to many physical, chemical and biological alterations. To describe and quantify these alterations, the concept of bioavailability has been developed. Bioavailability describes the rate and extent to which a drug enters systemic circulation after it is administered to a human or animal (Rowland and Tozer, 1995). The rate and extent of peroral absorption depend firstly on the chemical characteristics of the drug (water solubility, lipophilicity, particle size) and secondly on formulation variables like excipients and manufacturing procedures. In the early 1960´s, an awareness of and concern for bioavailability arose (Barret, 2004). It was observed that although two drug products contain the same molar doses of the active ingredient, safety and efficacy problems could emerge especially with low therapeutic index drugs like digoxin (Rowland and Tozer, 1995). Digoxin immediate-release formulations with different drug particle sizes had different bioavailability (Jounela et al. 1975). Differences in bioavailability of digoxin, even at the same dosage levels, can lead to one of the two extremes: either adverse effects or sub-therapeutic drug concentrations.

The Food and Drug Administration (FDA) introduced the first regulatory guidance for studying bioavailability and bioequivalency of new drug products in 1977. During the development of new drugs and drug products, several bioavailability and bioequivalency tests should be carried out. Batch size has to be scaled-up, different strengths need to be brought to market later on, or post-approval changes to the formulation need to be carried out. In all aforementioned cases, bioequivalency studies have typically been used to guarantee the therapeutic equivalency of the drug product. All bioavailability and bioequivalency studies are time-consuming and expensive, and *in vivo* studies involve a certain risk of adverse reactions among the healthy volunteers.

To reduce the need for *in vivo* bioequivalency studies, utilisation of *in vitro* dissolution tests as a surrogate for *in vivo* bioequivalence studies was introduced with the biopharmaceutical classification system (BCS) in 1995 (Amidon and Lennernäs et al., 1995). Since 1995, some scale-up and post-approval changes have been approved based on dissolution tests in the USA (FDA guidance, 1995). In 1997, the FDA published

regulatory guidances for *in vitro-in vivo* correlations (IVIVC), and regulatory authorities in Europe followed suit in 2000 (FDA guidance, 1997; EMEA, 2000). These guidelines allowed the use of dissolution tests and level A IVIVC models as surrogates for bioequivalence studies even when the drug product is significantly altered. In 2000, the FDA introduced regulatory guidance for BCS biowaivers (FDA guidance, 2000). The BCS biowaiver guidance includes detailed instructions for classification of drugs according to the BCS as well as requisites for waiving the bioequivalence studies in the case of major product changes or in the development of new generic immediate-release drug products. BCS I drugs with complete drug absorption and rapid dissolution were considered to be biowaiver candidates. Later, the European regulatory authority, EMEA, followed with a set of guidelines of similar principles (EMEA, 2002). IVIVC and BCS biowaiver guidelines have a remarkable potential for reducing the number of *in vivo* bioequivalence studies. However, fewer biowaiver-based new generic oral drug applications have been received than expected (Barends, 2005).

The World Health Organization (WHO) has actively utilised BCS biowaivers (WHO, 2006). New, cheap, effective and safe drug products are needed in developing countries. The Working Group on the Biopharmaceutical Classification System of the International Pharmaceutical Federation (FIP) has produced a series of publications in the Journal of Pharmaceutical Sciences. In these publications, the WHO list of essential drugs is inspected for new BCS biowaiver candidates. The BCS biowaiver evaluation for ranitidine presented in this thesis is part of this series.

In addition, with regulatory applications IVIVC and BCS biowaivers can be utilised in formulation development of new and generic oral drug products. The dissolution method can be used to guide formulation development, if a level A IVIVC is found. The selection of dissolution method and the utilization of *in vivo* data are critical steps in the development of a level A IVIVC model. The dissolution method should have an acceptable discrimination ability, i.e., formulations with statistically significant differences in pharmacokinetic parameters should have dissimilar dissolution profiles. The *in vitro-in vivo* relationship is typically determined using averaged *in vitro* and *in vivo* data. Many drugs have high pharmacokinetic variability and/or only a few subjects are involved in the *in vivo* studies. In these cases especially, development of an IVIVC model may fail if all relevant *in vivo* data cannot be utilised in the model. Subject-specific *in vivo* data,

uncertainty and variability related to the data and level A IVIVC model fitting can be described by integrating a stochastic approach into the level A IVIVC model. New criteria for selecting the dissolution method and utilisation of a stochastic Bayesian approach in level A IVIVC models are presented in this thesis.

BCS classification of new drug candidates or generic drugs gives information about the rate-limiting step for drug absorption, whether solubility or permeability limited. The BCS provides a practical first step to estimate oral drug absorption properties. However, the BCS system is a simplified approach to the complex absorption process, where physiological, physicochemical, drug- and formulation-related variables affect the rate and extent of drug absorption. This dynamic process cannot be estimated based on solubility, permeability, and dissolution properties. To take into account all variables simultaneously, a dynamic pharmacokinetic model is needed. In this thesis, intra- and inter-individual variability in gastric emptying, formulation type and drug elimination were taken into account for the first time in a pharmacokinetic model to predict bioequivalence of drugs and to suggest candidates for BCS biowaivers. BCS III drugs had special interest, because they have been suggested as biowaivers in many publications and they not seemed to be sensitive to dissolution rate differences, because permeability rate controls absorption (Blume and Schug, 1999; Yu et al. 2002; Cheng et al. 2004; Vogelpoel et al. 2004; Polli et al., 2004). On the other hand our own hypothesis, that C_{max} of part of the BCS I drugs were sensitive to dissolution rate differences needed to be verified.

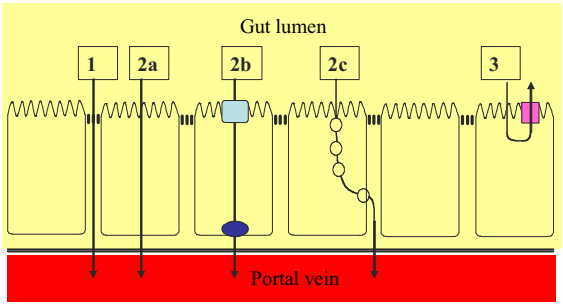
2 Literature Review

2.1 Oral drug absorption

Most drugs are administered orally, which is a convenient route for the patient. An identical drug dose is easy to administer repeatedly and the dosing is not dependent on administration technique, e.g. as with pulmonary drug treatment. However, after oral administration, the formulation and drug are exposed to a number of physicochemical, biopharmaceutical and pharmacokinetic factors before the drug reaches its site of action. During the first stage, the drug is released and dissolved from the solid dosage form to the gastrointestinal fluids and thereafter, the dissolved and solid drug particles are distributed to the gastrointestinal tract. The dissolved drug penetrates through the gut wall into the portal vein. Part of the drug may be metabolised in the gut lumen or the efflux proteins in the gut wall may efflux the drug back to the intestinal lumen. The drug flows from the portal vein to the liver, where first-pass metabolism takes place before the drug begins to circulate systemically. Via systemic circulation, the drug is distributed to the peripheral tissues and to the site of action. The parent drug and its metabolites are mainly excreted to the feces and urine.

Transport mechanisms in the drug absorption phase

One of the most critical steps in oral absorption is drug penetration through the gut wall. Drugs may permeate by passive diffusion or via active ATP dependent transport proteins (Fig. 1). Small and lipophilic drug molecules favour passive diffusion via a transcellular route, i.e., they permeate through the cell membranes of the enterocytes. Small hydrophilic molecules can permeate passively through the paracellular space between the epithelial cells. Carrier-mediated transcytosis is involved in transport of some vitamins.



1. Paracellular passive diffusion
2. Transcellular pathway
   2a. passive diffusion
   2b. carrier-mediated
   2c. receptor-mediated endocytosis
3. Carrier-mediated efflux pathway

Figure 1 Transport mechanisms of orally administered drugs.

Active transport mechanisms require specific binding of the drug as a substrate to the transporter protein in the cell membrane. There are many different transporters that are expressed in the gut lumen, and they can transport chemicals with a variety of molecular structures. There are several specific carrier systems for organic cationic and anionic molecules, amino acids and peptides. These transporters shuttle compounds from the apical side to the basolateral side of enterocytes i.e. from gut lumen towards the portal vein. These influx transport systems are involved in absorption of endogenous compounds like nutrients and hormones, and xenobiotics like drugs and prodrugs. Efflux proteins pump molecules from the apical side to the gut lumen or from the basolateral side to the portal vein. They protect organs from exogenes like drugs. The relative importance of active and passive permeation depends also on the rate of passive diffusion. Rapid passive diffusion may dominate the entire absorption process, and active transport may have a minor role. In contrast, peptides have negligible passive diffusion and, therefore, their oral absorption is dominated by active transport.

Saturable processes in the absorption phase

Active transport, both influx and efflux, and metabolism in the intestine can be capacity limited (Ritschel and Kearns, 1999). Absorption and metabolism of low doses can obey first-order kinetics, but at high doses the transport capacity may be exceeded, and then the

system follows Michaelis-Menten kinetics. This leads to nonlinear dose-dependent pharmacokinetics, and in addition transporter-related drug-drug interactions may take place. A nonlinear absorption phase may cause intra- and/or inter-individual variability in drug absorption. Substrates and inhibitors for certain influx and efflux proteins are found using *in vitro* methods like Caco-2 cell culture studies. In the context of transport proteins, the determination of *in vitro-in vivo* relationships is challenging. Different quantities of efflux and influx proteins are expressed in Caco-2 cells compared to enterocytes in the gut lumen, and functionality of the proteins may differ. Thus quantitative *in vivo* predictions cannot be easily obtained. Interactions can be studied *in vivo* by administering an inhibitor of a certain transport protein. The antitumour drug topotecan was administered with and without GF120918, an inhibitor of breast cancer resistance protein and P-glycoprotein (Kruijtzer et al., 2002). Apparent oral bioavailability increased from 40% to 97% when inhibitor was administered with topotecan. As a reference intravenously adminstration to a separate group of subjects was used.

The rate and extent of oral absorption

The rate and extent of oral drug absorption both determine the access of the drug to its site of action (Code of Federal Regulations, 2003). Drug absorption is characterised by pharmacokinetic parameters such as the area under the curve of drug concentration in plasma (AUC), maximum drug concentration in plasma (C_{max}), the time for maximum concentration (t_{max}), and the mean residence time (MRT). The absolute bioavailability for the oral dosage form of a drug is the ratio between the AUC values for oral versus intravenous administration of an identical drug dose. Relative bioavailability is the ratio of different formulations, e.g., immediate-release versus controlled-release tablet. Oral drug absorption can be described by an apparent absorption rate constant (K_a). It can be calculated based on MRT values after administration of an oral solution (os) and i.v. injection (i.v.) using the following equation (Ritcshel and Kearns, 1999):

(1) $$K_a = \frac{1}{MRT_{os} - MRT_{i.v.}}$$

Drug bioavailability is one of the most critical pharmacokinetic factors when new oral drugs are developed (Abrahamsson and Lennernäs, 2004). Pharmacologically potent compounds may be inactive in *vivo* if the drug does not reach the target cells at adequate

concentrations. Poor bioavailability may be due to low solubility and dissolution of the drug, degradation in the gastrointestinal tract, low permeability across the gut wall, efflux to the gut lumen or extensive first-pass metabolism.

High variability in drug absorption

Many drugs have acceptable concentration - time profiles and adequate bioavailability without formulation modifications, but have high inter- and/or intra-individual variability in drug concentrations. This can be due to variability in drug transit, distribution, dissolution, metabolism or permeability in the gastrointestinal tract, or differences in systemic kinetics i.e. distribution and elimination. For example, solubility and dissolution of acidic drugs is dependent on pH. Solubility is low and dissolution slow in the stomach, whereas high solubility and rapid dissolution may be achieved in the more alkaline conditions in the intestine. The main absorption site for drugs and nutrients is the small intestine, where the absorptive area is large (120 m^2) compared to stomach (0.11 m^2) (Avdeef, 2001). Gastric emptying of solid particles is a random, highly variable process. Gastric residence times of pellets with a size of 25 μm-14 mm may vary from 32 to 420 minutes (Hunter et al., 1982; Jonsson et al., 1983). As a result of slow dissolution in the stomach, variable gastric emptying rates and rapid dissolution in the intestine, for an acidic drug the available concentration at the site of drug absorption may be highly variable. The C_{max} of rapidly absorbed and/or eliminated drugs is especially sensitive to differences in the absorption phase (Kaus et al., 1999). To reduce high intra- and inter-individual variability in drug absorption, the drug should dissolve rapidly in the stomach or in the duodenum. In that case, a high concentration of dissolved drug is available at the absorption site in the small intestine. To study the interplay of many time-dependent variables in drug absorption, the pharmacokinetic simulation model may be a useful tool.

2.2 The gastrointestinal solubility and dissolution of drugs

Drug solubility and dissolution in the gastrointestinal tract are dependent on physiological factors such as composition, volume, and hydrodynamics of the contents in the gut lumen (Dressman et al., 1998). Stomach, intestine and colon have different values for pH, fluid volumes, contents, hydrodynamics, and residence times. The stomach is acidic in the fasting state (pH 1.4-2.1) but its pH is elevated in the fed state (pH 3-7) (Dressman et al.,

1990; Dressman et al., 1998). In the small intestine, the pH varies from 4.4 to 8.0 in the fasting and the fed states (Gray and Dressman, 1996). Bile salts are mainly secreted into the upper parts of the small intestine (Dressman et al., 1998). Bile salts and lecithin may lead to improved solubility and dissolution of low solubility drugs by forming mixed micelles in the fasting state. The volume of fluids in the fasting stomach is as little as 20-50 ml (Wilson and Washington, 1989; Dressman et al., 1998; Schiller et al., 2005) and in the jejunum and ileum it is 120-350 ml (Dillard et al., 1965). Mixing in the stomach and intestine is more vigorous in the fed than fasting state. In the fasting stomach there are long periods of little or no motor activity. About every two hours "house-keeping" waves empty the stomach. Gastric emptying is a highly variable process both in the fasting and fed states. Residence times of solid drug particles in the stomach can vary from a few minutes to half a day (Dressman et al, 1998). In the fed state, the composition of meals consumed and the size of the solid drug particles have an effect on the residence time. Nutrient fluids empty by a regular contraction pattern with a regular frequency and amplitude. Solid particles with a size range of 1-2 mm may be emptied with nutrient fluids. Residence time in the intestine is 3.3 hours and it does not depend on nutrient status (Davis et al., 1986; Yu et al., 1996).

High solubility drugs in rapidly dissolving immediate release formulations dissolve under variable conditions and active ingredients are mainly absorbed from the upper intestine. In the case of poorly soluble, poorly permeable drugs or controlled release formulations, the absorption may be slow, taking place over long segments of small intestine and colon. These drugs and products are sensitive to environmental factors such as physiological variables in the gastrointestinal tract (Dokoumetzidis and Macheras, 2006). For example, the release-controlling excipients in controlled-release formulations may be pH sensitive, as well as drug solubility. Thus gastrointestinal tract variables together with drug and formulation properties should be taken into account when an *in vitro* dissolution method is developed. Hydrodynamics and the complex composition of gastrointestinal fluids are the most challenging factors to be simulated in *in vitro* dissolution tests (Dressman et al., 1998; Dokoumetzidis and Macheras, 2006). *In vitro* solubility and dissolution of low solubility drugs in aqueous buffers can underestimate the *in vivo* solubility and dissolution (Dressman, 2007). Thus the selection of medium for low solubility drugs is challenging.

2.3 Controlled-release formulations

With rapidly eliminating drugs it may be difficult to achieve and maintain therapeutic drug concentrations. The average drug concentration in plasma at steady-state is dependent on the dosing frequency and dose size according to equation 2:

$$C_{ss} = \frac{F * Dose}{CL * \tau} \quad (2)$$

where C_{ss} is the average steady-state drug concentration in plasma, F is the bioavailability, CL (l/h) is the clearance rate, and τ (h) is the dosing interval (Rowland and Tozer, 1995). To reduce dosing frequency, controlled-release formulations may be needed. There are several formulation techniques available to produce controlled-release formulations, e.g. excipient components may dissolve slowly in the gastrointestinal fluids or an undissolved matrix may release the drug slowly.

The absorption window can be a critical factor when controlled-release formulations are developed (Davis, 2005). Some drugs which have an active transport mechanism or favour the paracellular absorption route have poor absorption from the colon (Lennernäs and Abrahamsson, 2004; Thombre, 2005). The extent of absorption in small intestine and colon is dependent on the BCS class of drug (Ungell et al., 1998). The *in vitro* Ussing chamber method was used to study drug absorption from rat intestinal and colonic tissues. Class I and II drugs were well absorbed from colon if the transport mechanism was passive diffusion, but Class III and IV drugs have significantly lower absorption from colon than from small intestine.

2.4 The Biopharmaceutical Classification System

2.4.1 Classifying drugs in BCS

In 1995, Amidon and coworkers introduced the biopharmaceutical classification system for orally administered drugs (Amidon et al., 1995). Solubility, dissolution and permeability are the main factors that affect the rate and extent of oral absorption. Based on these properties, drugs are placed into four classes (Fig. 2). BCS class I drugs have high solubility and permeability, class II have high permeability but low solubility, class

III have low permeability and high solubility and for class IV drugs both permeability and solubility are low. Drug dissolution is classified as rapid if more than 85% of the dose is dissolved in 30 minutes.

Detailed instructions for determining the BCS class of a drug are given in the regulatory guidelines (FDA guidance, 2000; EMEA, 2002). There are minor differences in methodology and application of BCS in the USA and Europe (Gupta et al., 2006). A drug substance has high solubility if the highest dose strength dissolves in 250 ml of aqueous buffer solution. According to the FDA guidance, solubility should be measured through a pH range of 1-7.5 and in the EMEA guidelines a pH range of 1-6.8 is required. Drug permeability is considered high if 90% or more of an orally administered drug is absorbed (FDA guidance, 2000). Linear and complete absorption indicates high permeability according to the EMEA guidelines (EMEA, 2002). Permeability can be determined based on *in vivo* mass balance studies or studies on absolute bioavailability (FDA guidance, 2000). Intestinal permeability methods *in vivo*, *in situ* or *in vitro* can be used. Animal models and/or *in vitro* methods can be used for passively transported drugs. Instructions in the EMEA guidelines are more non-specific than those in the FDA guidance. Therefore, the EMEA guidance is currently under revision to generate more precise definitions and instructions for BCS biowaivers (EMEA, 2007).

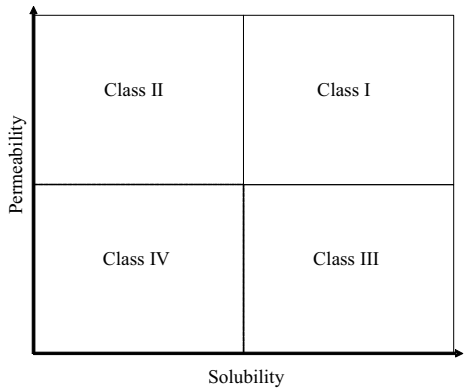


Figure 2 Biopharmaceutical classification of drugs.

2.4.2 Applications of BCS

BCS classification can be utilised in drug candidate selection at an early phase in drug development, during formulation development, and in regulatory applications (Lennernäs and Abrahamsson, 2005). The BCS class of a drug indicates the rate-limiting step for oral absorption: gastric emptying, dissolution or intestinal permeability (Fig. 3) (Amidon et al., 1995). In the early development phase, the permeability and solubility boundaries can be set as selection criteria for new drug candidates (Abrahamsson and Lennernäs, 2004). *In vitro* methods are utilised to measure solubility and permeability. Solubility is typically measured by the shake-flask method and permeability by Caco-2 cells.

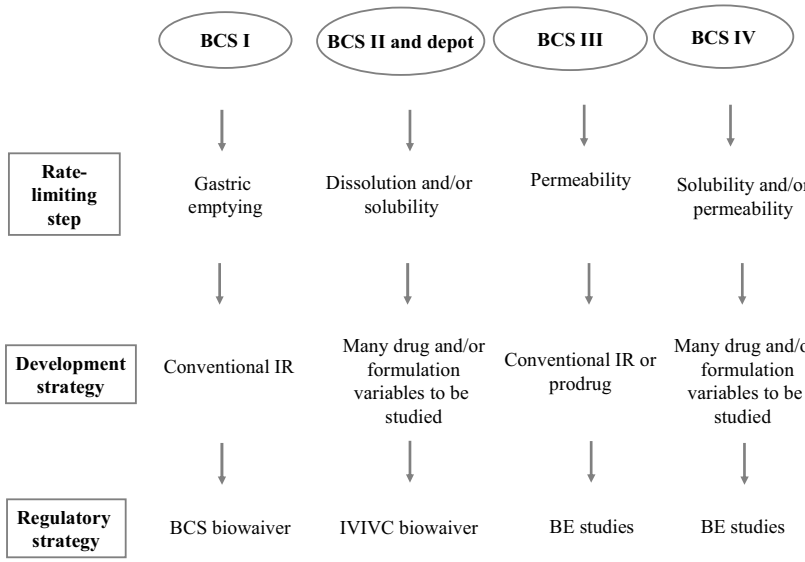


Figure 3 Application of the BCS in formulation development and appropriate regulatory strategies.

BCS I drugs

Gastric emptying of the dissolved drug is the rate-limiting step for oral absorption of class I drugs with rapid dissolution (Fig. 3). Class I drugs have favourable absorption properties, leading to rapid and complete absorption. Drug absorption can be mediated either by passive transcellular diffusion or by active transport. Even simple, conventional immediate-release formulation assures rapid and complete absorption for this class of drugs. Therefore, formulation development is fast and cheap unless other issues, such as stability or production problems, exist. IVIVCs cannot be found for immediate-release

formulations of class I drugs if dissolution is faster than gastric emptying. Thus, the dissolution method can be a simple and cheap quality control tool. However, if a BCS biowaiver is utilised in a regulatory application, dissolution should be tested in three different media representing the pH range of the gastrointestinal tract.

BCS II drugs

Dissolution controls absorption of class II drugs and a point-to-point relationship, i.e., level A IVIVC, can be found between *in vitro* dissolution and *in vivo* dissolution or absorption (Fig. 3). Like BCS I drugs, class II drugs have high permeability, and transport may be active or occur by passive transcellular diffusion. If absorption is limited by solubility or dissolution, it may be incomplete. Formulation development may be more challenging than for BCS I drugs if special tehniques and skills are utilised to enhance drug solubility or dissolution. For example, nanoparticles, microemulsion, cyclodextrins or lipid formulations can be used (Abrahamsson and Lennernäs, 2004; Lennernäs and Abrahamsson, 2005). *In vitro* dissolution method development also requires more time and a high level of knowledge if *in vitro* conditions are to mimic drug release and dissolution *in vivo*. Several pH values, agitation speeds, and different apparatuses should be tested. An appropriate method should discriminate critical formulation or manufacturing variables of the product affecting drug dissolution *in vivo*. If successful, a level A IVIVC may be proven and *in vitro* dissolution tests can be used as surrogates for *in vivo* bioavailability and bioequivalency studies.

BCS III drugs

Class III drugs have permeability limited absorption (Fig. 3). Incomplete absorption due to limited permeability can rarely be solved by formulation factors, because specific and non-toxic permeability enhancers are difficult to develop (Lennernäs and Abrahamsson, 2005). Instead, bioavailability may be increased by prodrug derivatization of the parent compound, improving drug distribution to the target tissue (Steffansen et al., 2004). The prodrug can be more lipophilic than the parent drug, faciliating trancellular passive diffusion or, alternatively, the prodrug can be designed to be a substrate for a transporter. The lipophilicity of the angiotensin-converting enzyme (ACE) inhibitors has been increased by using the ethyl ester, as for example was done with the prodrug of enalapril (Abrams et al., 1984; Todd and Heel, 1986; Beaumont et al., 2003). Bioavailability of the

parent compound is 3%, which improves to 36-44% for the ester prodrug. Valaciclovir, a prodrug of acyclovir, utilises an oligopeptide transporter and has 5 times better bioavailability than acyclovir (Han et al., 1998).

In many cases, permeability is high enough to achieve therapeutic drug concentrations in plasma. Then conventional immediate-release formulation is a good choice. For example, the BCS III drugs ranitidine and cimetidine in immediate-release tablets have bioavailabilities of 50-60% (Bogues et al., 1980; Garg et al., 1981; Jantratid et al., 2006). In many cases, the prodrug approach is not needed if therapeutic drug concentrations are achieved with the parent drug and with simple and cheap conventional formulations.

An IVIVC can not be found for BCS III drugs when permeability is the rate-limiting step for absorption. The role of the dissolution method is to act as a quality control tool to ensure batch-to-batch consistency. Dissolution method development is thus easier for such class III drugs than for class II drugs or controlled-release products.

BCS IV drugs

Class IV drugs have low solubility and permeability. The rate-limiting step in drug absorption can be solubility, dissolution or permeability (Fig. 3). The fraction of absorbed drug dose may be low and highly variable because class IV drugs have problems in solubility and permeability. Formulation and dissolution methods may be similar to those for class II drugs if dissolution is the rate-limiting factor. For permeability-limited absorption, class IV drugs may be developed like class III drugs. Some class IV drugs may be unsuitable for oral administration if the fraction absorbed is too low and oral absorption is highly variable. However, the tolerated level of variability depends on the indication and therapeutic index of the drug.

2.4.3 BCS biowaivers

Bioequivalence studies may be replaced by BCS or IVIVC biowaivers (Fig. 3). For a biowaiver, the *in vitro* dissolution study may be used as a surrogate for *in vivo* bioequivalence studies. The first regulatory guidance utilising the BCS was published in 1995, when the FDA published a guidance for scale-up and post-approval changes (SUPAC) of drug products (FDA guidance, 1995). Requirements were based on the BCS

class of the drug in question and the significance of the change. SUPACs are divided in three levels: level 1 changes are unlikely to have any detectable impact on formulation quality and performance; level 2 changes could have a significant impact; and at level 3 the changes are likely to have impact on bioavailability. Level 2 changes can be applied for based on presentation of data for rapid dissolution for BCS I drug products, and presentation of dissolution profile comparisons for BCS class II or III drugs. Level 3 changes have to be justified by *in vivo* studies if a level A IVIVC is not found.

BCS biowaiver guidelines were introduced in 2000 by the FDA and in 2002 by EMEA (FDA guidance, 2000; EMEA, 2002). *In vivo* bioequivalency studies may be replaced by *in vitro* studies for BCS I drugs if test and reference drug products have similar dissolution profiles (Fig 2.). In the USA, drug products should also have rapid dissolution, i.e. > 85% should dissolve in 30 minutes at three different pH values (range 1-6.8) (FDA guidance, 2000). To be a biowaiver candidate, the application should include scientific justification, e.g. that the drug does not have a narrow therapeutic index and that all excipients are well-know (FDA guidance, 2000; EMEA, 2002). Importantly, the excipients should not have interactions with the pharmacokinetics of the drug component. Atypically large amounts of known excipients or new excipients in the drug formulation require additional information in the regulatory application.

Currently, BCS I drugs are defined as biowaiver candidates because the rate limiting step for absorption is gastric emptying of the dissolved drug. Minor differences in the dissolution rates therefore have negligible effects on C_{max} and AUC. Conventional immediate-release dosage forms should behave more like oral solutions. However, the rate-limiting step for absorption can be a complex combination of several factors, i.e., gastric emptying, solubility, and dissolution can all contribute to the absorption rate of the drug. Despite its merits, the BCS classification is an oversimplification of the dynamic process of oral drug absorption.

2.4.4 BCS biowaiver extensions

During the time period spanning 2000-2007, regulatory agencies have received fewer BCS biowaiver applications than expected. This is the case especially for new generic drug products (Polli et al., 2004; Barends, 2005; Gupta et al., 2006, EMEA, 2007). There are a few published revisions to methodologies for classifying drugs in the BCS, and extension

of biowaivers to acidic class II and class III drugs has been suggested. Hopefully these will lead to BCS guideline revisions and increase BCS biowaiver applications.

Methodology revisions

It has been suggested that the solubility boundary for biowaiver candidates should be narrowed from pH 1-7.5 to 1-6.8 and the fraction of the dose absorbed should be reduced from 90% to 85% (Yu et al., 2002; Polli et al., 2004). Currently, a drug product is considered rapidly dissolving if more than 85% dissolves in 30 minutes. A new criterion of 60 minutes for the dissolving time has been suggested (Polli et al., 2004). For acidic drugs, solubility tests in conditions mimicking small intestinal pH may be more appropriate than tests performed at pH 7.5 (Yazdanian et al., 2004). To classify drug solubility, the solubility is measured in aqueous buffer using a volume of 250 ml. It has been suggested that the volume should be increased from 250 ml to 500 ml and that surfactants may be added to the medium (Polli et al., 2004). However, these revisions need experimental verification before they can used.

BCS II drugs

BCS II drugs have not been accepted as biowaiver candidates by the regulatory agencies, but acidic BCS II drugs have been suggested as possible candidates for biowaivers in scientific publications (Rinaki et al., 2004; Yazdanian et al., 2004). Those publications criticise the current biowaiver guidelines, which are based on equilibrium solubility and dissolution tests, and in which the dynamic nature of drug absorption is not taken into account. Acidic BCS II drugs have low solubility only in the stomach, while solubility in the small intestine is high and the fraction of the dose absorbed can be > 0.9. The extent of oral drug absorption (i.e. AUC) may not be sensitive to minor dissolution rate differences under the alkaline conditions in the small intestine. In contrast, the rate of oral absorption (i.e. C_{max}) may be sensitive to differences in the dissolution rates, as was pointed out in simulation studies (Kaus et al., 1999). Solubility and dissolution of acidic BCS II drugs are site dependent, i.e., solubility is low in the acidic stomach and high in the alkaline small intestine. As discussed previously, gastric emptying of solid drugs is a highly variable process, since house-keeping waves occur every 1.3-2 hours (Wilson and Washington, 1989). Thus, drug concentrations at the absorption site may vary and minor dissolution rate differences may cause fluctuations in C_{max} values.

BCS III drugs

For BCS III drugs, biowaivers can not be utilised in regulatory applications in the USA and Europe, but in a report recently published by the WHO, BCS III drugs were accepted as biowaiver candidates (WHO, 2006). There are many scientific papers published where class III drugs are recommended as a biowaiver candidates (Blume and Schug, 1999; Yu et al. 2002; Cheng et al. 2004; Vogelpoel et al. 2004; Polli et al., 2004). For this BCS class, the permeability rate controls absorption and the bioavailability is more dependent on the drug (permeability) than on the formulation (dissolution). The test and reference products will be bioequivalent if absorption is permeability rate limited. Class III drugs may be even better biowaiver candidates than class I drugs, if the effects of excipients on gastrointestinal transit time and permeability can be excluded (Blume and Schug, 1999). BCS III drugs which are substrates of efflux proteins and/or have extensive metabolism in the intestine should not be accepted as biowaiver candidates. These saturable mechanisms are dependent on drug concentration and thus in some cases even minor differences in the concentration can lead to changes in the rate and/or extent of absorption.

2.5 *In vitro-in vivo* correlation

2.5.1 Level A, B and C IVIVC

Drug dissolution is the rate-limiting step in absorption for BCS II drugs and controlled release formulations (Fig 3). Therefore, *in vitro* dissolution of the drug correlates with its *in vivo* absorption. To predict *in vivo* input rate, the dissolution method should discriminate between the variables of drug substance, product and/or manufacturing method that affect the rate and extent of drug release and dissolution. In the most successful case, *in vitro* dissolution conditions that mimic *in vivo* dissolution may be found.

For reasons of clarity, three levels - A, B and C - have been defined for IVIVC (FDA guidance, 1997). Level A IVIVC is the highest level and it has the most extensive applications. It represents a point-to-point relationship between *in vitro* dissolution and *in vivo* input rate. Only in the case of level A IVIVC can *in vitro* dissolution be used as a surrogate for *in vivo* bioequivalence studies, i.e., the level A IVIVC model and dissolution

method can be used in biowaivers. Level B and C IVIVC models have less predictive power a than level A IVIVC model, because C_{max}, T_{max} or MRT values can be the same for different formulations. In level B IVIVC, parameters are based on statistical moment analysis. Typically, MRT or mean dissolution time *in vivo* ($MDT_{in\ vivo}$) is compared to the mean dissolution time *in vitro* ($MDT_{in\ vitro}$). Level C IVIVC represents single-point correlation between one dissolution time point and one pharmacokinetic parameter. For example, the time for 80% drug release can be correlated with C_{max}.

2.5.2 Development of a level A IVIVC model

To develop and validate a level A IVIVC model, two or three different formulations should be studied *in vitro* and *in vivo* (FDA guidance, 1997). Typically, the qualitative composition of drug products is the same, but the release-controlling variable(s), e.g., the amount of excipient, or a property of the drug substance such as particle size, is varied. To develop a discriminative *in vitro* dissolution method, several method variables together with formulation variables are studied, e.g., different pH values, dissolution apparatuses and agitation speeds.

Development of a level A IVIVC model includes several steps. In the first step, the *in vivo* input profile of the drug from different formulations is calculated from drug concentrations in plasma (Fig. 4). To separate drug input from drug distribution and elimination, model-dependent approaches, such as Wagner-Nelson and Loo-Riegelman, or model independent procedures, based on numerical deconvolution, may be utilised (Wagner and Nelson, 1964; Loo and Riegelman, 1968; Cutler, 1978; Veng-Pedersen, 1992). The Wagner-Nelson protocol for a one-compartment model is the simplest method, because an i.v., oral solution or immediate-release formulation is not needed as a reference. Loo-Riegelman two-compartment and numerical deconvolution methods require administration of a reference formulation. In step 1, the parameters that describe drug input rate, drug distribution and/or elimination are determined. In the model dependent approaches, the distribution and elimination rate constants describe pharmacokinetics after absorption. In the numerical deconvolution approach, the drug unit impulse response function describes distribution and elimination phases, respectively.

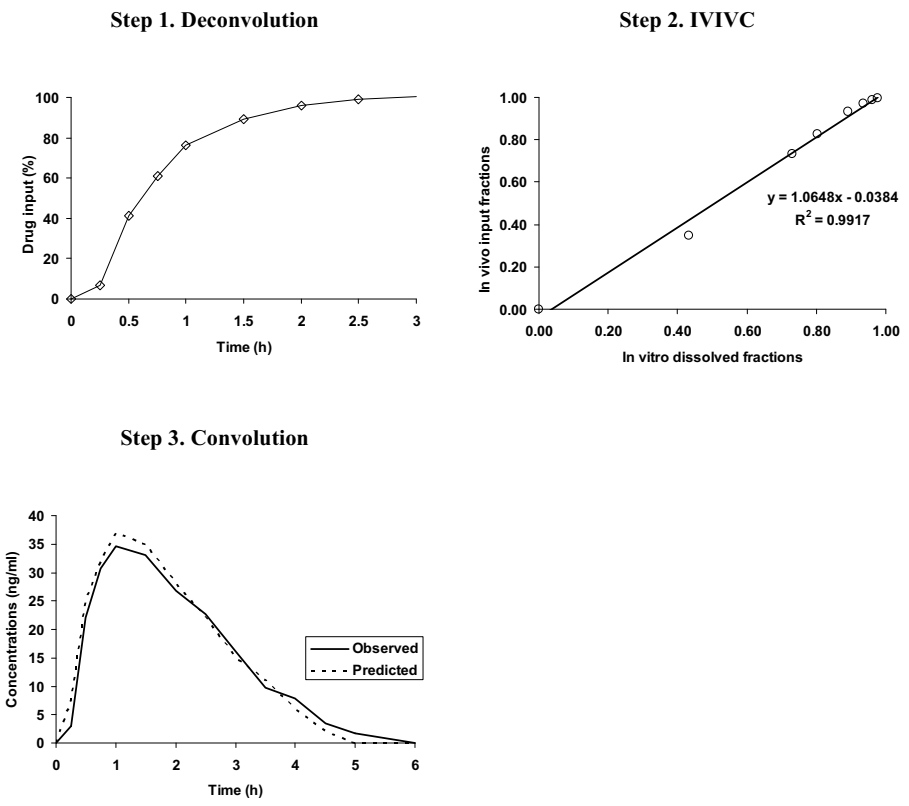


Figure 4 The main steps in the development of a level A IVIVC model.

In step 2, the relationship between *in vitro* dissolution and the *in vivo* drug input profile is determined (Fig. 4). Either a linear or nonlinear relationship may be found. In some cases, time-scaling of *in vitro* data must be used, because *in vitro* dissolution and *in vivo* input may follow the same kinetics but still have different time-scales (Brockmeier et al., 1983; FDA guidance, 1997). The time-scaling factor should be the same for all formulations if an IVIVC at level A is sought.

In step 3, plasma drug concentration profiles are predicted and compared to the observed time courses for different formulations (Fig. 4). To generate predicted time courses, the drug input profile is predicted based on *in vitro* dissolution data and the *in vitro-in vivo* relationship generated in step 2. In the convolution process, the predicted drug input and parameters describing drug distribution and/or elimination phases are combined in order to get predicted time courses. This procedure, which includes steps 1-3, is called two-stage

deconvolution. Alternatively, a drug input profile based on *in vitro* dissolution data can be solved together with parameters describing systemic pharmacokinetics, i.e. distribution and elimination. This approach is called direct convolution.

Internal and external predictability of a level A IVIVC model

The predictability of a level A IVIVC model is tested by calculating percent prediction error (%PE):

$$\%PE = \left[\frac{(observed - predicted)}{observed} \right] \times 100 \quad (3)$$

where the observed and predicted values of C_{max} and AUC are used. A level A IVIVC model has acceptable predictability if the average percent prediction errors for the formulation series are less than 10% (FDA guidance, 1997; EMEA, 2000). In addition, the percent prediction error for each formulation should not exceed 15%. Validity of IVIVC models can be tested internally with data used to define the IVIVC, or externally with data that was not used for model development. External predictability has tighter limits for percent prediction error (10%) and it is recommended especially for narrow therapeutic index drugs.

2.5.3 Applications of IVIVC models

The main objective of developing and validating IVIVC models is to establish conditions for dissolution tests which can be used as surrogates for relative bioavailability or bioequivalence studies. Several bioavailability and bioequivalence studies are conducted during the development process of a new drug product. The manufacturing scale of the drug product is increased from laboratory scale via a pivotal scale to the final production scale, and typically the regulatory application for a new drug includes several strengths (Lennernäs and Abrahamsson, 2005). BCS II and controlled-release drugs also need formulation optimisation studies in order to achieve target drug concentrations in plasma. Another application of IVIVC models is for drug products already on the market which need some alteration, for example because of changes in manufacturing site, equipment, process or formulation. In general, if an IVIVC biowaiver can not be utilised, several formulations and strengths need to be studied *in vivo* leading to higher costs and longer

development times.

Level B and C IVIVC models do not have extensive possibilities for use in biowaiver applications, because they do not represent point-to-point relationships between *in vitro* and *in vivo* parameters. Different types of formulations can have the same AUC, C_{max} or T_{max} values, even if the shapes of the concentration profiles are different. Thus, level B or C IVIVC models cannot be used to replace relative bioavailability or bioequivalence studies.

The possibilities for successful development and use of a level A IVIVC model depend on the quality of the *in vivo* data i.e. how many subjects are involved in the study, whether the study involves administration of reference drug, and how many different formulations are studied. The most important thing is to use a logical series of formulations in the *in vivo* study in order to validate the IVIVC model.

Averaged IVIVC models

There are many published studies where an averaged level A IVIVC model is developed for one immediate-release formulation of BCS II drug (Nicolaides et al., 2001), for one modified-release formulation (Mahayani et al., 2000; Modi et al., 2000; Dalton et al., 2001) or for a logical series of modified-release formulations (Eddington et al., 1998; Sirisuth and Eddington, 2000; Veng-Pedersen et al., 2000; Sirisuth et al., 2002a; Sirisuth and Eddington, 2002b). Averaged IVIVC models for one or two formulation(s) have limited possibilities for use in biowaver applications because only minor changes to the manufacturing process or formulation may be applied for based on an IVIVC biowaiver (FDA guidance, 1997). Averaged IVIVC models for logical series of formulations have more extensive possibilities for use in biowaiver applications because interpolation is possible within the formulation series. Formulations utilised in IVIVC model development have the same qualitative composition and the same release- and dissolution-controlling factors, but different dissolution rates. Then, approval of even major changes, e.g. changes to release-controlling excipients, may be applied for. However, the change should be within the range of release-controlling excipients for the established correlation.

Stochastic IVIVC models

The information from the original *in vitro* and especially *in vivo* data may be lost when an IVIVC model is constructed using the averaged approach. In many cases inter- and intra-subject variability in pharmacokinetics is much greater than the variability between formulations (Mauger and Chinchilli, 1997). Thus the averaged level A IVIVC model may be misleading or model development may fail. A stochastic model, representing data variability as well as uncertainty related to both the subjects and the ability of the IVIVC model to describe observed data, may be integrated into a level A IVIVC model. Most of the stochastic IVIVC models are developed for one formulation, not for a formulation series (Bigorra et al., 1997; Mauger and Chinchilli, 1997; Verotta, 1997; Dunne et al., 1999; O'Hara et al., 2001; Pitsiu et al., 2001; Qiu et al., 2003). In these studies mixed effect approaches have been combined with IVIVC models to describe variability related to the *in vivo* data. In this type of level A IVIVC model, all subjects are modelled simultaneously and, depending on the *in vivo* study design, intra- and/or inter-individual variability related to the subjects can be taken into account in the predictions. In most published examples, the deconvolution step is involved i.e. comparison of *in vitro* dissolution and *in vivo* input curves (Bigorra et al., 1997; Verotta, 1997; Dunne et al., 1999; Qiu et al., 2003). A convolution approach together with mixed effect modelling has been presented in one publication, where concentration profiles of one formulation were predicted for each subject (Pitsiu et al., 2001). There is one published example of development of a mixed effects IVIVC model for a formulation series (Mauger and Chinchilli, 1997). In that study, the model construction and validation were not presented in sufficient detail. Thus there is need for transparent stochastic IVIVC models for formulation series, which can utilised both during drug development and in the post-approval period. Level A IVIVC model can be validated, when it is constructed to the formulation series. The Bayesian approach enable the use of subject-specific in vivo data and probably better predictability is achieved than with corresponding averaged level A IVIVC models.

2.6 Regulatory applications of biowaivers

In many cases *in vivo* bioequivalence studies can be replaced by *in vitro* dissolution studies in regulatory applications. The application can be based on BCS or IVIVC biowaivers or simply on a dissolution profile comparison. BCS biowaivers have the most extensive possibilities for use in drug approval applications, because they can be used in applications for approval even of new generic drug without *in vivo* bioequivalence studies. A BCS biowaiver is acceptable for BCS class I drugs formulated as rapidly dissolving immediate-release products (FDA guidance, 2000; EMEA, 2002). In that case, the application may be based on *in vitro* dissolution and permeability data together with scientific justification of linear pharmacokinetics, a proof that the drug does not have a narrow thepapeutic index and that the excipients do not have pharmacokinetic interactions with the drug. Both BCS and IVIVC biowaivers can be utilised for changes in drug product composition, manufacturing site or production method. An IVIVC biowaiver can be used for BCS II drugs in immediate-release dosage forms and for controlled-release formulations.

Approval of new, lower strengths or some minor changes to the drug product can be applied for based on the dissolution profile comparison without IVIVC or BCS biowaivers. Then, to guarantee bioequivalency, the new dose strength or new formulation should have a similar dissolution profile to that of the drug formulation already on the market. IVIVC and BCS biowaivers can be utilised even for major changes which are known to have some effect on drug absorption. However, the classification of changes as minor or major is different in the USA and Europe, and there are even differences in such classification between national European agencies.

2.7 Quantitative absorption models

In the BCS, the dynamic nature of drug solubility, dissolution and permeability is not taken into account. The BCS can be used to estimate oral drug absorption, but a quantitative approach is more accurate and informative. Maximum absorbable dose, fraction of dose absorbed or rate-limiting step for drug absorption can be estimated when *in silico*, *in vitro* or *in vivo* parameters such as solubility, dissolution, and permeability are gathered together. In addition, relevant physiological factors, like small intestinal transit

time, liquid volumes, and pH values in the gastrointestinal tract, can be taken into account in a quantitative absorption model.

2.7.1 Prediction of fraction of dose absorbed and maximum absorbable dose

Maximum absorbable dose (MAD)

MAD is a useful parameter for new drug candidates. In addition to solubility and permeability, transit time in small intestine and water volume are used to calculate MAD (Curatalo, 1998) using equation 4:

(4) $$MAD = S \times K_a \times V \times T$$

where S is drug solubility, K_a is the absorption rate constant, V is the intake water volume (250 ml) and T is the transit time in small intestine (199 min). MAD can also be calculated using the following equation (Sun et al., 2004):

(5) $$MAD = P_{eff} \times S \times A_{eff} \times T$$

where P_{eff} is drug permeability in human intestine and A_{eff} is the effective absorption surface area. P_{eff} can be replaced with *in vitro* Caco2 permeability data using the following equation (Sun et al., 2004):

(6) $$P_{eff} = 10^{(0.6532 \log P_{eff,Caco2} - 0.3036)}$$

Mass balance approach

The mass balance approach can be used to predict fraction of dose absorbed (F_{abs}) and to estimate critical factors affecting the extent of drug absorption (Amidon et al., 1995). However, plasma concentration profiles cannot be predicted with this kind of steady-state model. Three dimensionless-parameters, i.e. dose number (Do), dissolution number (Dn) and absorption number (An), are utilised in predictions (Amidon et al., 1995; Löbenberg and Amidon, 2000). Dose number is equal to the Q value (Rinaki et al., 2003a) according to equation 7:

(7) $$Do = Q = \frac{Dose}{S * V}$$

where S is solubility and V is liquid volume in stomach or intestine (250 ml). High solubility drugs have Q≤1 and low solubility drugs have Q>1. Absorption number is

(8) $$An = \frac{T_{res}}{T_{abs}}$$

where T_{res} is mean small intestinal transit time and T_{abs} is mean absorption time, which can also defined as follows:

(9) $$T_{abs} = \frac{R}{P_{eff}}$$

where R is radius of the tube (small intestine) and P_{eff} is effective permeability. For drugs with complete absorption, the value of An is more than one, and incomplete absorption gives a value of An that is less than 1.

Dissolution numbers is

(10) $$Dn = \frac{T_{res}}{T_{diss}}$$

where T_{diss} is the mean dissolution time, which can also defined as follows:

(11) $$T_{diss} = \frac{r_0^{\,2}\rho}{3DS}$$

where r_o is the initial particle radius, ρ is density of the particle and D is the diffusion coefficient. To obtain parameters for calculating mean dissolution time, theoretical assumptions should made. However, from *in vitro* dissolution data, mean dissolution time can be determined e.g. with a the Weibull function. Drugs with complete dissolution have a Dn value greater than one, and incomplete dissolution lead to a Dn value of less than 1.

Quantitative BCS

F_{abs} can be predicted semi-quantitatively with a quantitative BCS model (Rinaki et al., 2003b). The method is based on a tube model, where drug particles move down the tube, dissolve in the intestinal fluids and permeate through the intestinal wall. Mean transit time in the intestine (MITT), mean dissolution time (MDT), mean absorption time (MAT) and mean effective time (MET=MAT+MDT) describe these processes. Drugs can be categorised, for example, as fully absorbed ($F_{abs} \geq 0.95$) when MET<< MITT, and as having dissolution limited absorption when MET>>MAT and therefore MDT>>MAT.

2.7.2 A dynamic model to predict drug concentration profile

The compartmental absorption and transit model (CAT) is a dynamic model by which both F_{abs} and drug plasma concentration profiles can be predicted (Yu et al., 1996; Agoram et al., 2001). In the CAT model, drug absorption and gastrointestinal transit processes are described with nine compartments (Yu et al., 1996; Yu and Amidon; 1999b). Stomach and colon are described as one compartment each and the small intestine as seven compartments. In the CAT model, gastrointestinal tract physiology (gastric emptying, small intestinal transit and distribution), physico-chemical (permeability, solubility), and pharmaceutical (release and dissolution) factors can be easily taken into account. Pharmacokinetic parameters describing drug distribution and/or elimination phases can be integrated into the CAT model in order to predict the concentration-time profile (Agoram et al., 2001). Both linear non-saturable and non-linear saturable processes can be taken into account and simulated with the CAT model. However, the biggest challenge is to scale the *in vitro* data describing drug dissolution, transport or metabolism to correspond to *in vivo* processes in humans.

2.7.3 Comparison of different methods to predict the rate-limiting step of absorption

BCS classes I and II include diverse drugs for which the absorption may depend on gastric emptying, solubility and/or dissolution. Quantitative kinetic models may be useful for identification of the rate limiting steps. Then, variables related to the drug substance, drug product and gastrointestinal tract physiology can be taken into account simultaneously,

and the most critical factors in drug absorption may be determined.

The rate-limiting step for absorption of digoxin, a BCS I drug, has been estimated using several quantitative absorption models. Based on the BCS classification, the rate-limiting step for absorption of digoxin from immediate-release formulations should be gastric emptying. Absorption of digoxin was studied using the mass balance approach (Löbenberg and Amidon, 2000). Dose number, absorption number and dissolution number were plotted to find critical factors determining the extent of drug absorption. The extent of absorption of a high permeability drug with a relative low dose number, like digoxin (Do=0.08), is sensitive to the to dissolution rate differences. Thus, absorption of digoxin can be increased with faster dissolving products like micronized drug subtance. Quantitative BCS was utilised for digoxin (Rinaki et al., 2003b). Mean dissolution time was longer than the mean absorption time, indicating dissolution-dependent drug absorption. An integrated drug absorption model (that resembles the CAT model) was used to predict particle size effects on digoxin absorption (Yu, 1999a). The model showed that absorption was dependent on particle size, and it was able to predict the empirical *in vivo* data. It can be concluded, based on all three quantitative absorption models, that the rate-limiting step for digoxin absorption is dissolution instead of gastric emptying (Yu, 1999a; Löbenberg and Amidon, 2000; Rinaki et al., 2003b). This is in agreement with the empirical findings (Jounela et al., 1975). Relative bioavailabilities from immediate-release tablets with 0.25 mg of digoxin were 96, 78 and 37% for particle sizes of 7, 13 and 102 μm, respectively. In this study, oral solution was used as the reference. Quantitative absorption models are needed for characterization of the rate-limiting step for absorption and to guide formulation and regulatory strategies for oral dosage forms.

3 Aims of the study

The overall aim of the study was to generate and utilise pharmacokinetic and IVIVC models to facilitate formulation development and preparation of regulatory applications. The specific goals of the study are:

1. To develop and select a predictive dissolution method for a level A IVIVC model of levosimendan modified-release capsules.

2. To develop a level A IVIVC model using a Bayesian approach for a levosimendan modified-release formulation series by utilising subject-specific *in vivo* data. The final goal of the model was to obtain *in vivo* predictions as probability distributions.

3. To evaluate ranitidine, a BCS III compound, as a potential biowaiver drug.

4. To build a pharmacokinetic simulation model to evaluate the relative risks of bioinequivalence and to suggest candidates for biowaivers among BCS I-IV drugs.

4 Materials and methods

4.1 IVIVC studies (I, II)

4.1.1 Development of a biorelevant dissolution method for levosimendan (I)

In vitro and *in vivo* data

In the formulation series (F, G, H and I), the dissolution and absorption of levosimendan was slightly extended by formulation factors i.e. the amount of release controlling excipients increased from formulation F to I. The qualitative composition of the formulations was the same. The *in vitro* dissolution rate of levosimendan was studied using the basket method at pH 5.8 or 7.4. Levosimendan concentrations in the samples were analysed by UV spectrophotometer.

Levosimendan absorption from the oral formulations was studied in nine healthy volunteers. Drug concentrations in plasma were determined by reversed-phase high performance liquid chromatography using UV detection (Karlsson et al., 1997).

Data analysis

In order to select a biorelevant and discriminative dissolution method for the modified release formulations, *in vitro* dissolution and *in vivo* bioavailability data were analysed by several methods. To test *in vivo* relevance of different dissolution methods, level B and C IVIVC models were constructed for the formulation series, and a level A IVIVC model for one of the formulations. To test how dissolution methods discriminate between the formulations, the dissolution profiles were compared using similarity factors (f_2) (Moore and Flanner, 1996). Pharmacokinetic parameters, C_{max}, AUC, T_{max} and MRT were calculated. The differences in pharmacokinetic parameters were determined with statistical analysis of variance (ANOVA). In all calculations, commercial software was utilised.

4.1.2 Development of a stochastic IVIVC model for levosimendan formulations (II)

In vitro and *in vivo* data

In the development of the stochastic level A IVIVC model, the Bayesian approach was combined with the pharmacokinetic one-compartment model. *In vitro* and *in vivo* data from publication I and two additional levosimendan absorption studies, as well as related dissolution data, were used in the model construction and predictability tests (II, Fig.1). In this study, formulation codes A, B, C and D were used for formulations F, G, H and I, respectively.

One-compartment modelling

In vitro dissolution and *in vivo* absorption data were combined in a convolution model modified from a pharmacokinetic one-compartment model (Wagner and Nelson, 1964).

$$
\begin{aligned}
&t > t_{lag}: \\
&C(t) = \frac{K_a F D_{po}}{V_c (K_a - k_{10})} \cdot \left\{ e^{-k_{10} \cdot (t - t_{lag})} - e^{-K_a \cdot (t - t_{lag})} \right\} \\
&t \le t_{lag}: \\
&C(t) = 0
\end{aligned}
\tag{12}
$$

where K_a is the apparent absorption rate constant, F is bioavailability, 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} is the lag time of drug absorption. The one-compartment model was modified by replacing the K_a with a dissolution rate constant (K_d) and a time-scaling factor (a).

The stochastic IVIVC model

In order to build the stochastic IVIVC model, subject-specific absorption data were used. A Bayesian approach was combined with the modified one-compartment model. The most important part of the stochastic IVIVC model is the likelihood function that defines the connection between the modified one-compartment model parameters (a, F/V_c, K_{10}, t_{lag}) and the experimental data (Gelman et al, 1995). The explanatory variable was K_d, the first order rate of *in vitro* dissolution. That parameter was measured at pH 5.8 using a rotation

speed of 100/min for each formulation. A log-normal noise model was used to explain the deviations of levosimendan concentrations in plasma from the compartment model. As a result of simulations, the marginal posterior distributions for modified one-compartment model parameters were obtained. By combining posterior distribution of model parameters with the dissolution data, we predicted the drug concentration profiles in plasma for each formulation. The concentration-time profiles were presented as maximum values of the posterior distribution (MAP) and as 95% probability intervals. The predicted parameters (AUC, C_{max}) were presented as histograms and MAP. In all calculations, MATLAB (MathWorks Inc., USA) was utilised.

4.2 Risk analysis studies of bioequivalence and biowaivers (III,IV)

4.2.1 Evaluation of ranitidine as a biowaiver candidate (III)

Relevant literature data was collected for ranitidine immediate-release dosage forms. The data included solubility and dissolution values, permeability, pharmacokinetic parameters, therapeutic use, and therapeutic window. Furthermore, data about excipient interactions and problems of bioavailability and/or bioequivalence was collected. The Caplus, Ipa and Medline databases were utilised. Solubility data from the literature did not cover the entire physiological pH range. Therefore, solubility determinations were carried out. Ranitidine suspensions were shaken in buffer solutions covering the relevant pH range and the concentrations of dissolved ranitidine were analysed by high performance liquid chromatography.

Literature data was compared to the current BCS biowaiver requirements defined in the guidelines (FDA guidance, 2000; EMEA, 2002) and in scientific papers suggesting extensions to the current requirements. BCS classification of ranitidine was done based on experimental solubility determinations and *in vitro* Caco-2 studies published in the literature. Risks related to the bioequivalence of different immediate-release dosage forms were estimated based on dissolution properties and excipients used in immediate-release formulations on the market in Germany, the Netherlands and Finland.

4.2.2 Simulated risk analysis for bioequivalence and biowaivers (IV)

Computer simulations were carried out to probe the sensitivity of C_{max} and AUC to dissolution rate. In all cases, oral solution was compared to immediate release formulations and the relative changes in C_{max} and AUC were simulated.

The model structure

Gastric emptying, drug dissolution, transit in the intestine and absorption were all incorporated into the compartment absorption and transit model (CAT) (Fig. 5) (Yu et al., 1996). The stomach was described as a single compartment and the intestine as seven compartments in series. In the simulations, the drug was allowed to dissolve both in the stomach and intestine, but absorption took place only in the intestine. Solid and dissolved drug were transferred from the stomach to the intestine. Thereafter, the transit and distribution of the undissolved and dissolved drug in the intestine were similar. After its first-order absorption, the drug was assumed to obey one-compartment kinetics with a first-order rate of elimination from plasma. The model was constructed using STELLA software (ISEE systems, Lebanon, USA).

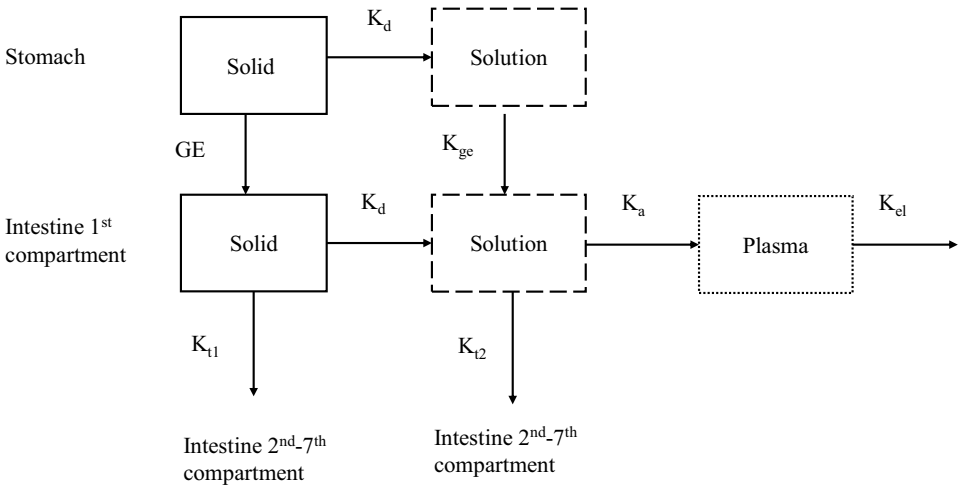


Figure 5 The structure of the CAT model and parameters used in the model: GE is gastric emptying for solid drugs, K_{ge} is the gastric emptying rate constant for dissolved drug, K_d is the dissolution rate constant, K_{t1} and K_{t2} are transit rate constants for solid and dissolved drug respectively, K_a is the absorption rate constant and K_{el} is the elimination rate constant. The drug transits, dissolves and absorbs identically from all 7 compartments of the intestine.

Gastrointestinal parameters

Literature data from imaging studies was analysed to obtain parameters describing the rate and kinetics of gastric emptying after administration of oral solution and solid formulations with single or multiple units (IV, Table 1). Oral solution empties from the stomach according to first-order kinetics, multiple unit formulations use zero-order kinetics, and single-unit formulations empty as a single rapid bolus. Fast, average and slow gastric emptying rates were calculated based on the literature data for multiple and single unit formulations.

Rates of drug dissolution, absorption and elimination

The rate of drug dissolution in the model was set at either 2 h^{-1} or 4 h^{-1}, and these hypothetical formulations were compared to the performance of drug solution. A dissolution rate constant of 4 h^{-1} means that more than 85% of the drug is dissolved in 30 minutes, whereas at K_d = 2 h^{-1} more than 85% of the drug is dissolved in 60 minutes.

In the simulations, the rates of drug absorption (K_a) had a wide range (0.1-8 h^{-1}) that spans the scale of both high and low permeability drugs of the BCS (IV, Table 2). Absorption rate constants of 1.2-8 h^{-1} enable complete drug absorption (>90%). Low permeability drugs have absorption rate constants of 0.1-0.8 h^{-1} and they show incomplete absorption. The range of elimination rate constants was 0.014-0.9 h^{-1}. There were 366 clinically used drugs in this range as of 1999 (Ritschel and Kearns, 1999).

The parameter values that were used in the simulations overlapped all four BCS classes. In the simulations, the BCS II and IV drugs were assumed to be acids, i.e., they had low solubility in the stomach and high solubility in the intestine.

Risk assessment of bioequivalence studies and potential for biowaivers

The AUC and C_{max} values were simulated and the ratios of these values for oral solid formulation over those for oral solution (oral solid formulations/oral solution) were calculated for each set of parameter combinations. Multiple and single unit dosage forms were studied using fast, average and slow gastric emptying rates in the fasting state. For bioequivalence and biowaiver criteria we suggest here a maximum of 10% difference in AUC and C_{max} between the solid dosage forms and oral solution.

5 Results

5.1 IVIVC for levosimendan modified-release capsules (I, II)

5.1.1 Dissolution and absorption properties of different formulations (I, II)

In vitro dissolution properties

Levosimendan dissolution was rapid at pH 7.4 from all formulations (F, G and H) when the speed of rotation was 100 rpm (Fig. 6). At a rotation speed of 50 rpm, the dissolution rate decreased. Formulation G had slower dissolution than formulation H. At pH 5.8, higher amounts of rate-controlling polymers caused slower dissolution rates (Fig. 7). The best discrimination was achieved at pH 5.8 and a rotation speed of 50 rpm. In this case the similarity factors (f_2) were less than 50, indicating dissimilar dissolution profiles among all formulations. Formulations F, G, and H had similar dissolution profiles at pH 5.8 and a rotation speed of 100 rpm, but formulation I differed from all the others.

In vivo absorption properties

The pharmacokinetic parameters C_{max}, AUC, T_{max} and MRT for formulations F through I are presented in Table I. An increased amount of alginic acid and hydroxypropylmethylcellulose decreased the rate of drug absorption from the formulations. There was a rank-order correlation between the amount of rate-controlling excipients and the values of MRT and C_{max}. Differences in the C_{max} values were statistically significant ($p<0.05$) between formulations F vs. H, F vs. I, and G vs. I. Based on T_{max} and MRT values, drug absorption from formulation I was significantly slower than from all the others, whereas formulations F, G and H did not statistically significantly differ from each other.

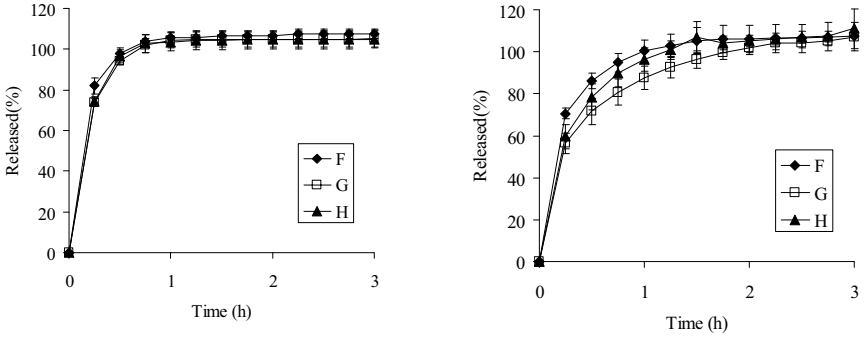


Figure 6 Dissolution of levosimendan from modified-release capsules (F-H): ♦ = F, □ = G and ▲ = H. Dissolution conditions: pH 7.4 and a rotation speed of 100 rpm (left) and 50 rpm (right), means ± SD, n = 5-6.

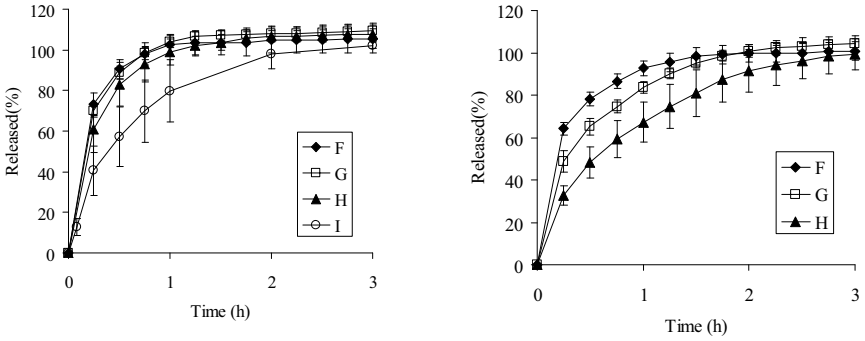


Figure 7 Dissolution of levosimendan from modified-release capsules (F-I): ♦ = F, □ = G, ▲ = H and ○ = I. Dissolution conditions: pH 5.8 and a rotation speed of 100 rpm (left) and 50 rpm (right), means ± SD, n = 5-6.

Table I Pharmacokinetic parameters of modified-release capsules of levosimendan after a single dose of 2 mg (means ± SD, n=9).

	F	G	H	I
C_{max} (ng/ml)	64.4±26.3	55.5±23.0	45.3±20.6	36.9±18.6
T_{max} (h)	1.0±0.3	1.4±0.9	1.3±0.7	2.0*±0.4
$AUC_{0\text{-}12h}$ (ng*h/ml)	107±49	99±47	86±39	95±47
MRT (h)	1.6±0.3	1.8±0.6	1.9±0.7	2.4*±0.3

*Statistically different, $p < 0.05$, compared to formulations F, G and H

5.1.2 Selection of dissolution method for a level A IVIVC model (I)

In vitro dissolution at pH 7.4

At pH 7.4 and a rotation speed of 100 rpm, the discrimination ability was acceptable when similarity factors were compared to the variance in the T_{max} and MRT of formulations F, G and H (Table II). However, level B or C IVIVC was not achieved with this method. Although the level B correlation coefficient was high (0.94), the IVIVC model was not reliable (Fig. 8). A pH of 7.4 with a rotation speed of 50 rpm was not acceptable because there was no rank order correlation between MRT and C_{max} vs. dissolution rate. Neither level B nor level C *in vitro-in vivo* correlation was achieved.

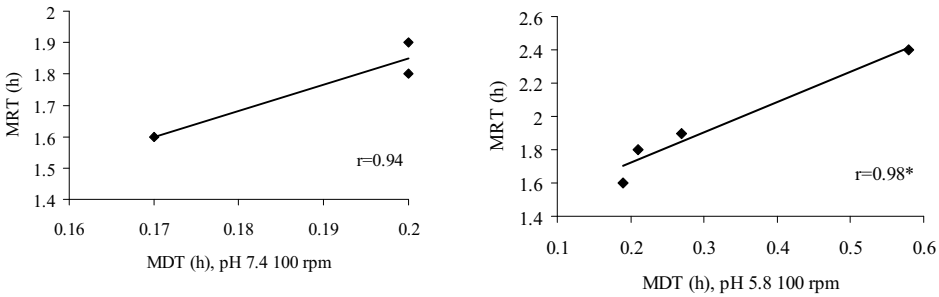
Table II Comparison of dissolution profiles to pharmacokinetic parameters and correlation coefficients of level B and C IVIVC.

	pH 7.4, 50 rpm	pH 7.4 100 rpm	pH 5.8 50 rpm	pH 5.8 100 rpm
F_2 vs. ANOVA	-	+	-	+
Level C IVIVC	0.52	0.71	1*	0.85
Level B IVIVC	0.74	0.94	0.93	0.98*

+ acceptable discrimination, - unacceptable discrimination, *statistically significant $p<0.05$

In vitro dissolution at pH 5.8

Dissolution at pH 5.8 and a rotation speed of 50 rpm was over-discriminating between the formulations. Formulations F, G and H had dissimilar dissolution profiles, although MRT and T_{max} values did not differ (Tables I and II). However, a level C IVIVC was achieved with this method. At a rotation speed of 100 rpm, formulations F, G and H had similar dissolution profiles, but formulation I was different. The discrimination ability of this dissolution method was acceptable, because T_{max} and MRT values of formulation I were statistically significantly different from all other formulations. An acceptable level B IVIVC was achieved with this dissolution method (Fig. 8).



*statistically significant $p<0.05$

Figure 8 Level B IVIVC for modified-release capsules at pH 7.4 and 5.8 with a rotation speed of 100 rpm. *In vivo* mean residence time (MRT) and *in vitro* mean dissolution time (MDT) were used in level B IVIVC.

5.1.3 A stochastic level A IVIVC model for the formulation series (II)

The averaged level A IVIVC model

In vitro and *in vivo* data for formulations A to D were utilised in the construction of the averaged level A IVIVC model. Formulations E and F were used to test external predictability. The external prediction errors for C_{max} were 15% and 70% for formulations E and F, respectively. The averaged level A IVIVC model failed for modified-release formulations. An alternative approach was used to obtain a level A IVIVC model.

Modified one-compartment model parameters

A stochastic Bayesian approach was combined with a modified one-compartment model in order to utilise subject-specific *in vivo* data and to obtain predictions as probability distributions. Posterior distributions of modified one-compartment model parameters and the dissolution data were used to predict pharmacokinetic parameters and concentration time profiles for formulations A, B, C, D, E and F (Fig. 9, Fig. 10). The distributions for parameters F/V_c and k_{10} were quite narrow and uni-modal, indicating sufficiency of bioavailability data for IVIVC model construction. Parameter *a* was less than one because *in vitro* dissolution was faster than *in vivo* dissolution from the capsules. Histograms of distributions of the parameter t_{lag} were concentrated differently, indicating that the use of

individual t_{lag} values for each test subject was reasonable (not presented).

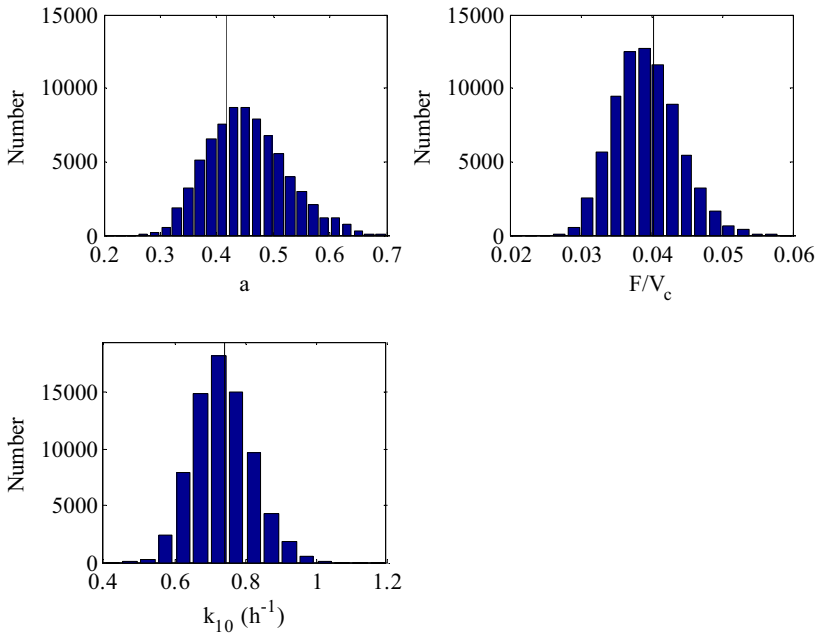


Figure 9 Histograms of marginal posterior distributions (number of simulated points =75 000) of parameter *a*, F/V_c, k_{10} and MAP values (vertical line).

Predicted vs. observed time courses

Predicted concentration-time profiles of levosimendan are presented with posterior probability interval curves and maximum values of the posterior probability distribution (MAP) (Fig. 10). The observed concentration profiles of formulations A to D had a slightly different shape from the predicted profiles. The observed mean values were not always within the predicted 95% posterior probability intervals. Concentration profiles for formulations E and F, which were not used in the model construction, were predicted well, as the shapes of the observed and the predicted profiles were similar. The observed mean values were within the predicted 95% posterior probability intervals, except for a single mean value for formulation F.

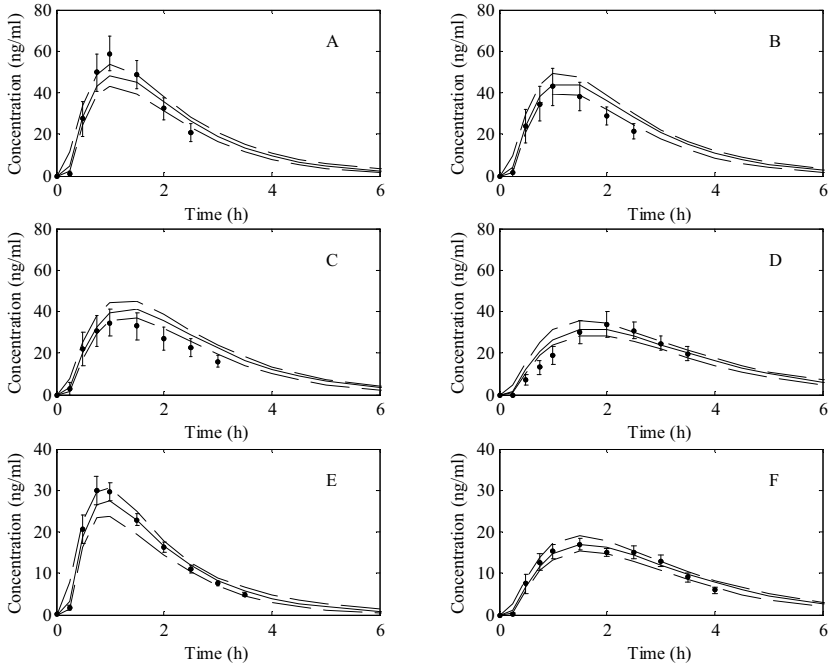


Figure 10 Observed average values for the plasma concentration time profiles ± SEM (dots with error bars), predicted 95% posterior probability interval curves for average levosimendan concentrations in plasma (dashed lines) and MAP values (continuous line) for modified-release capsules A-F.

5.2 Ranitidine as a biowaiver candidate (III)

Pharmacokinetic properties

The oral bioavailability of ranitidine is 50-60% (Bogues et al., 1980; Garg et al., 1981; Chau et al., 1982; Martin et al., 1982; Garg et al., 1983). Half-life of elimination is 1.7-2.1 h after an i.v. dose (Bogues et al., 1980; Garg et al., 1981; Mignon et al., 1982; van Hecken et al., 1982). The elimination rate constants are 0.3-0.4 h^{-1}.

The absorption of ranitidine after oral administration of different doses is linear (Mignon et al., 1982). Permeability across a Caco-2 cell monolayer was low, 1 to 20 x 10^{-7} (Gan et al., 1993; Walter et al., 1996; Collet et al., 1999; Lee and Thakker, 1999; Takamatsu et al., 2001; Yazdanian et al., 2004) and it increased at lower calcium concentrations (Gan et al.,

1993). Colonic absorption of ranitidine is poor (Williams et al., 1992; Pithavala et al., 1998). These *in vitro* and *in vivo* observations refer to a paracellular transport mechanism. The solubility was high - more than 550 mg/ml - in the pH range of 1 to 7.4. Ranitidine is a BCS III drug having low permeability and high solubility.

Immediate-release dosage form performance

The effect of excipients on the *in vitro* permeability of BCS III drugs, including ranitidine, has been studied (Aungst, 2000; Rege et al., 2001). Lactose, hydroxypropylmethyl cellulose, docusate sodium, ethylene diamine tetracetic acid (EDTA), propylene glycol and polyethylene glycol (PEG) 400 did not affect the Caco-2 permeability of ranitidine. Some other excipients, such as sodium lauryl sulphate, sodium caprate, deoxycholate, glycocholate, taurodihydrofusidate and palmitoylcarnitine, which open tight junctions, may affect the absorption of drugs transported via the paracellular route. High concentrations of osmotically active excipients such as sodium acid pyrophosphate and PEG 400 may shorten the small intestinal transit time and thus reduce the bioavailability of ranitidine (Koch et al. 1993; Basit et al., 2002; Schulze et al., 2003).

The dissolution of immediate-release ranitidine products is rapid, i.e. >85% dissolves in 30 minutes when water or 0.1 N HCl is used as a dissolution medium (III, Table 3) (Polli, 1997; Ali et al., 1998; Cappola, 2001; Yu et al., 2002). Based on similarity factors, all formulations did not have similar dissolution profiles. Similarity of dissolution profiles does not seem to be a critical factor because dissimilar but rapidly dissolving products were bioequivalent (Piscitelli et al., 1995; Polli, 1997). Simulation studies of ranitidine and atenolol, also a BCS III drug, showed that solid dosage forms were bioequivalent with oral solution if more than 85% dissolved in 1.5 hours (Kaus et al., 1999). In this study less than 20% differences in C_{max} and AUC were accepted to prove bioequivalency. Simulations are supported by an experimental *in vivo* study in which immediate-release tablets of metformin, also a BCS III drug with a paracellular transport mechanism, were shown to have absorption similar to modified-release tablets (Balan et al., 2001; Cheng et al., 2004). More than 85% was dissolved in 2 hours from modified-release formulations.

5.3 Evaluation of bioequivalence risks and BCS biowaivers by pharmacokinetic modelling (IV)

Rapid *in vivo* dissolution ($K_d = 4\ h^{-1}$)

Less than 10% differences in C_{max} and AUC values were obtained in the simulations when solid dosage forms ($K_d = 4\ h^{-1}$) were compared to oral solution (Fig. 11). C_{max} ratios of BCS III drugs were closer to 1.0 than the C_{max} ratios of BCS I drugs, although only BCS I are currently accepted for biowaivers.

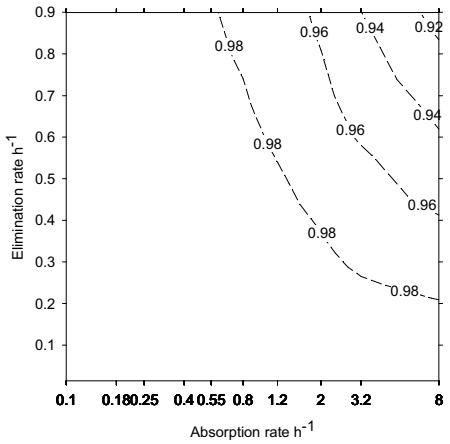


Figure 11 C_{max} ratios ($C_{max\ tablet}/C_{max\ oral\ solution}$) of BCS I and III drugs in single unit formulation with an average gastric emptying rate. Absorption rates of 1.2-8 h^{-1} correspond to high permeability and 0.1-0.8 h^{-1} to low permeability compounds.

BCS I ($K_d = 2\ h^{-1}$)

The rates of drug absorption and elimination affected the relative differences between the C_{max} values of solid dosage forms and oral solution (Fig. 12). As the absorption and elimination rates increased, C_{max} values for the oral dosage form deviated increasingly from the values for oral solution. BCS I drugs with rapid absorption and elimination had more than 10% differences in C_{max} values. The greatest difference, 25%, was observed with a single unit formulation when gastric emptying was slow. In contrast, the C_{max} ratio was nearly constant (0.95), and independent of the absorption rate, formulation and physiology related variables when the elimination rate constant was less than 0.2 h^{-1}. BCS I drugs in a single unit formulation were more sensitive to gastric emptying rate

differences than multiple unit formulations. AUC values had less than 5% differences at all gastric emptying rates and with both formulation types. AUC was dependent only on absorption rate, and differences between solid dosage forms and oral solution slightly increased when absorption rate decreased.

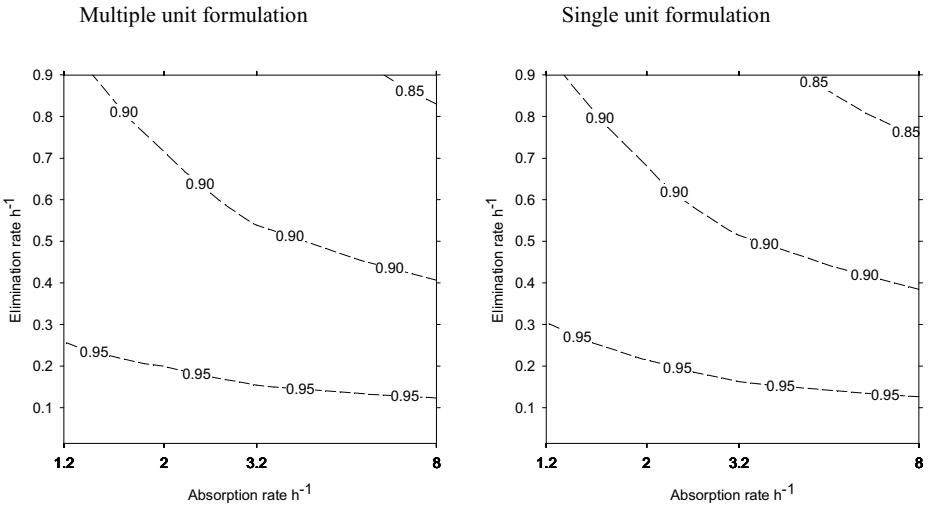


Figure 12 C_{max} ratios ($C_{max\ tablet}/C_{max\ oral\ solution}$) of BCS I drugs in multiple and single unit formulations with an average gastric emptying rate. Absorption rates of 1.2-8 h^{-1} correspond to high permeability compounds.

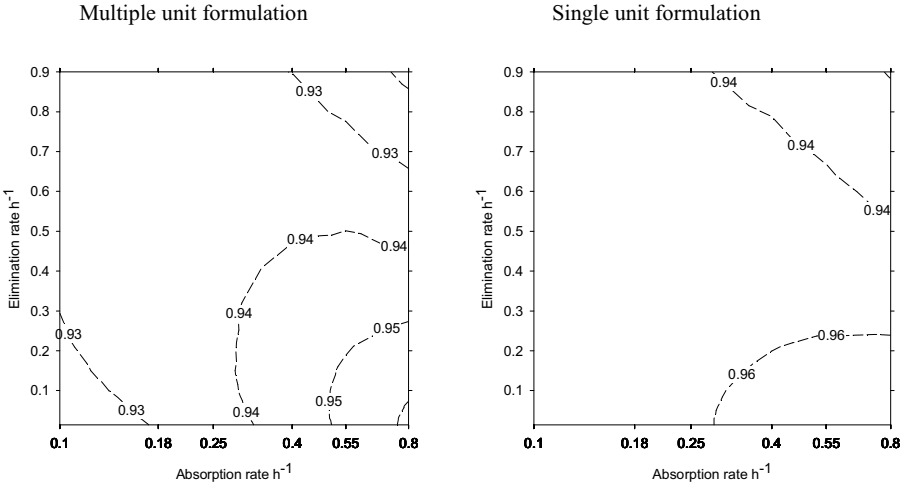


Figure 13 C_{max} ratios ($C_{max\ tablet}/C_{max\ oral\ solution}$) of BCS III drugs in multiple and single unit formulations with an average gastric emptying rate. Absorption rates of 0.8-0.1 h^{-1} correspond to low permeability compounds.

BCS III ($K_d = 2\ h^{-1}$)

BCS III drugs were less sensitive to dissolution rate, gastric emptying and formulation type differences than BCS I drugs. Observed differences in AUC and C_{max} values were less than 10% and thus all BCS III drugs fulfil the biowaiver criteria (Fig. 13).

BCS II and IV drugs ($K_d = 2\ h^{-1}$)

Solubility in the stomach, formulation type and gastric emptying rates were all critical factors for C_{max} differences of BCS II and IV drugs. In these two classes, clear and general trends in C_{max} were not seen and some biowaivers may be found on a case by case basis.

6 Discussion

6.1 Evaluation of ranitidine as a BCS biowaiver candidate (III)

Literature data has been collected and evaluated to suggest potential BCS biowaiver compounds in the World Health Organisation (WHO) list of essential drugs. Our publication III for ranitidine is part of a series of publications organised by the Working Group on the Biopharmaceutical Classification System of the International Pharmaceutical Federation (FIP).

Ranitidine is a BCS III drug having high solubility and low permeability. Oral drug absorption of different doses is linear (Mignon et al., 1982). The main absorption site is small intestine, and the permeability mechanism seems to be paracellular passive diffusion (Williams et al., 1992; Gan et al., 1993; Pithavala et al., 1998). Immediate-release ranitidine tablets have rapid dissolution (>85% in 30 minutes) in water and 0.1 N HCl (Polli, 1997; Ali et al., 1998; Cappola, 2001; Yu et al., 2002). The rate-limiting step for absorption of ranitidine is permeability, and minor differences in dissolution rate have no effect on absorption; thus, rapidly dissolving ranitidine formulations are bioequivalent (Piscitelli et al., 1995; Polli, 1997). Simulation results for ranitidine and *in vivo* study results for metformin, also a BCS III drug, indicate that rapid dissolution is not necessary for bioequivalence (Kaus et al, 1999; Balan et al, 2001). In those simulations, an oral solution of ranitidine was predicted to be bioequivalent with a solid dosage form having dissolution as slow as >85% dissolved in 1.5 hours. An immediate-release metformin formulation had a similar *in vivo* input profile to that of a controlled-release formulation. More than 85% dissolved in 2 hours from the controlled-release formulation. The dissolution profile of the immediate-release metformin formulation was not presented in these publications. The data show that rapid dissolution or similarity of dissolution profiles is not a critical factor for bioequivalency of ranitidine dosage forms. Therefore, ranitidine immediate-release tablets can be proposed as a biowaiver candidate, although ranitidine is BCS III drug.

Our study together with the Vogelpoel et al. (2004) study of atenolol are the first in which a BCS III drug is proposed to be suitable for a biowaiver based on an extensive drug-specific evaluation of data from the scientific literature. Currently, only BCS I drugs are accepted for biowaivers, but extension of biowaivers to the BCS III drug class has been discussed (Blume and Schug, 1999; Dressman et al, 2001; Yu et al, 2002).

Dissolution test can be used as a surrogate for bioequivalence studies of ranitidine immediate-release products, if the effects of excipients on gastrointestinal transit and permeability can be excluded. Some osmotically active excipients at high concentrations decreased the small intestinal transit time and reduced the bioavailability of ranitidine (Koch et al, 1993; Basit et al., 2002; Schulze et al., 2003). However, amounts as high as those used in these studies (1-10 g) are not used in immediate-release formulations. More attention should be paid to the excipients, which may open tight junctions and may increase absorption of paracellularly transported drugs (Aungst, 2000; Rege et al., 2001). However, many commonly used excipients have no effect on *in vitro* Caco-2 permeability (Rege et al., 2001). Expertise in properties of excipients, mechanisms of transport systems and *in vitro* permeability study methods is needed when determining the effects of excipients on permeation of BCS III drugs like ranitidine.

To be on the safe side when applying for a biowaiver for ranitidine, the test and reference formulations have to comply with the requirements for "rapidly dissolving" according to the FDA guidelines, i.e. not less than 85% of the labelled amount dissolving within 30 min in 0.1 N HCl, in pH 4.5 buffer and in pH 6.8 buffer.

6.2 Potential use during the drug development process of the models developed

The dissolution test, IVIVC and pharmacokinetic models developed here can be utilised to estimate oral drug absorption properties during the different phases of the drug development process (Fig. 14). Drug input profile and time course can be simulated and predicted for drugs from all BCS classes and for different dosage forms (oral solution → controlled release formulations). Level A IVIVC models are restricted to the BCS II drugs and controlled release formulations.

In the drug discovery phase, three essential *in vitro* parameters - permeability, solubility and dissolution - can be easily used to determine BCS class and maximum absorbable dose. These analyses give information about the extent of absorption and the rate limiting step of drug absorption. However, to get more complete estimates of drug absorption, a dynamic pharmacokinetic simulation model, e.g. CAT, should be utilised already during the drug discovery phase. The *in vivo* input profile can be simulated when permeability, solubility and dissolution parameters are combined with known properties of the gastrointestinal tract. The most critical factors that affect drug absorption can be estimated, and different types of formulation strategies can be evaluated, e.g., to estimate the need for dissolution, solubility and/or permeability enhancers.

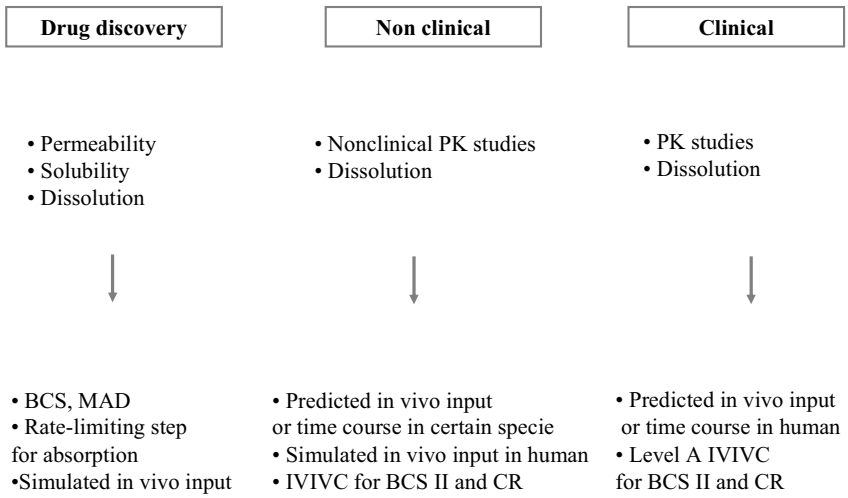


Figure 14 Simulating the rate and extent of oral drug absorption during the drug development process.

In the non-clinical study phase, pharmacokinetic data from various species is available. Again, permeability, solubility and dissolution parameters are combined with known physiological properties of the gastrointestinal tracts of certain species of test animals. Pharmacokinetic data on the distribution and elimination phases is now available, so concentration profiles can be predicted. The CAT model can be connected with the one compartment model to describe drug elimination, or with the two compartment model to describe drug distribution and elimination phases. Predicted and observed input or concentration profiles are compared. Scaling factors between *in vitro* and *in vivo* data can

be determined. In order to make simulations in human more reliable, a pharmacokinetic model should have predictive power for some test animal species. There are differences in the gastrointestinal physiology in animal vs. human, and the quantities of active transport proteins and metabolizing enzymes may vary. Thus quantitative predictions based on non-clinical data can not be made, but the critical factors affecting drug absorption, like low solubility and dissolution or low passive permeability, can be evaluated. For BCS II and controlled-release formulations, a level A IVIVC in some test animal species may be found. An IVIVC model can be utilised in formulation and dissolution method development.

In the clinical study phase, the CAT model is updated with human pharmacokinetic data. Then the predicted and observed inputs or concentration profiles in human can be compared. Scaling factors between *in vitro* and *in vivo* data are determined for human. Depending on the properties of the drug and quality of the *in vitro* and *in vivo* data, the possibilities for utilisation of the CAT model differ. In the most successful case, the plasma drug concentration profile can be predicted based on *in vitro* parameters describing the absorption phase and *in vivo* parameters describing the distribution and elimination phases. For BCS II and controlled-release formulations, a level A IVIVC model may be constructed and even validated. The early and extensive use of pharmacokinetic simulations and IVIVC models shortens the drug development period, economises on resources and improves drug pharmaceutical and biopharmaceutical quality.

Commercial pharmacokinetic modelling software

Commercial programs, e.g. IDEA and Gastroplus, can be utilised to simulate and predict oral drug absorption in different development phases for drug products. *In silico*, *in vitro* and *in vivo* data from test animals species can be used to predict absorption properties (Parrot and Lavé, 2002). However, these programs are not transparent; the whole structure of the model and the parameter values are not available. The major aim in the simulation is to combine many variables that are related to gastrointestinal tract physiology and drug and formulation properties, and to learn which are the most critical factors affecting drug absorption. Only a self-constructed transparent model enables an interactive learning process. Stella software is user-friendly and can be used to construct compartment models like CAT. The Stella program used with the CAT model is easy to update with whatever

new data becomes available during the drug development process.

6.2.1 The potential for utilisation of level A IVIVC models in formulation development (I,II)

For BCS II drugs and for controlled release formulations, drug release is the rate-limiting step in absorption. Thus a relationship between *in vitro* dissolution and *in vivo* input should be found. The development of IVIVC models is a dynamic process that should start early in the development phase and continue until the drug comes to market (Fig. 15) (Devane, 1997). In the ideal case, level A IVIVC model development and utilisation should proceed as described below.

In the first stage, prototype formulations are developed, i.e. different approaches to enhance drug dissolution and solubility (BCS II, IV) or to extend drug release are tested. Formulations can have different release mechanisms due to e.g. different compositions and manufacturing methods. The *in vitro* dissolution method should be discriminative for formulation variables and take into account physicochemical properties of the drug. Typically various dissolution media, apparatuses and agitation speeds are tested. The most promising prototype formulations are tested in animals and/or humans. A level A IVIVC model can be developed when *in vivo* pharmacokinetic and dissolution data is available for different formulations. All prototype formulations probably have different IVIVC models, i.e. models based on different dissolution methods and time-scale factors between *in vitro* and *in vivo* data. Level A IVIVC models are formulation specific and thus cannot be used to predict *in vivo* input or concentrations in plasma for formulations that are different from those studied *in vivo*. However, if two or three prototype formulations having the same qualitative composition have been studied, a target absorption profile may be achieved and a validated level A IVIVC model can be developed. Then the most promising formulation and dissolution method can be selected for further development. In the less successful case, a target absorption profile is not achieved and new formulations have to be developed and studied *in vitro* and *in vivo*.

In the second stage, after prototype formulations have been developed, the batch size is scaled-up and formulation is optimised. To get a validated level A IVIVC model, at least two (three are recommended) different formulations should be studied *in vivo* (FDA guidance, 1997). The exception is formulations in which *in vitro* dissolution is

independent of dissolution test conditions e.g. pH and agitation speed. Then, one formulation is enough for IVIVC model development and validation. Formulations having *in vitro* dissolution that is dependent on test conditions should have the same qualitative composition, but the amount of rate-controlling excipients are varied. The dissolution method has to be sensitive to formulation-specific variables, like the amount of rate-controlling excipient and manufacturing process variables. Formulation, process and dissolution method optimisation studies together with a validated level A IVIVC model give a good understanding of the release mechanism of formulations and the most critical variables affecting drug release and dissolution. In some cases, the target formulation can be intermediate between two formulations studied *in vivo*. Then the validated level A IVIVC model can be used for optimisation of the final formulation. Only the most promising formulation(s) should be studied *in vivo*. For the final formulation, dissolution specifications can be set based on the IVIVC (FDA guidance, 1997; Piscitelli and Young, 1997).

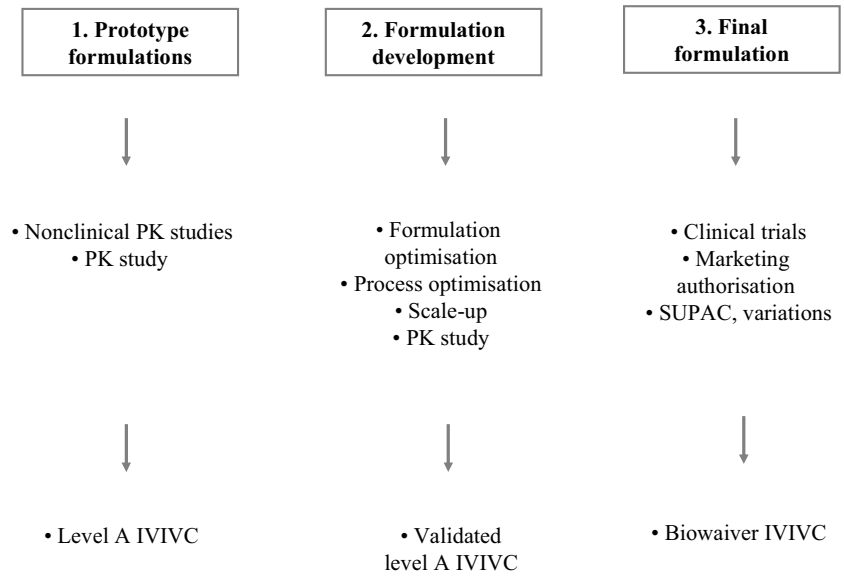


Figure 15 The IVIVC model development process for BCS II drugs and controlled-release products during drug development.

In the third stage, a validated level A IVIVC model together with *in vitro* dissolution data can be used for a biowaiver when the drug product is modified. The drug product can be under clinical studies or already on the market. Validated level A IVIVC models guide formulation and manufacturing method revision, and *in vivo* bioequivalence studies are not needed in the regulatory application.

The selection of dissolution method for a level A IVIVC (I)

The selection of a dissolution method for a level A IVIVC model is a critical step in the formulation development process of BCS II drugs and controlled-release formulations. To be successful, physicochemical, pharmaceutical, biopharmaceutical and pharmacokinetic properties of the drug and drug product together with gastrointestinal physiology should be taken into account. A pharmacokinetic simulation model may be a useful tool to combine information from different sources.

Levosimendan is a BCS I drug having rapid absorption and elimination phases (Sandell et al., 1995; Sundberg et al., 1998). Thus, drug concentration levels in plasma will fluctuate, and it must be administered in an immediate-release formulation several times a day. To avoid this, a series of modified-release formulations were developed. The qualitative composition of four formulations was similar, but the amount of the rate-controlling excipients hydroxypropylmethylcellulose and alginic acid increased from formulations F to I.

To develop a level A IVIVC for these modified-release formulations, several dissolution methods were tested. The rotation speed of the basket was either 50 or 100, and pH values of 5.8 and 7.4 were selected because they are around the pKa value of levosimendan (6.26) and they represented pH values of the upper (pH 5.8) and lower parts (pH 7.4) of the intestine. Bioavailability and dissolution data was used to study *in vitro-in vivo* relationships. Level B and C IVIVC models were generated and similarity factors of the dissolution profiles were compared to the differences in pharmacokinetic parameters using ANOVA. Based on the level B and C IVIVC models, the pH 5.8 dissolution methods were promising. A rotation speed of 100 rpm was the best choice and had adequate discriminating power. A rotation speed of 50 rpm was too discriminating, because even formulations without statistically significant differences in T_{max} and MRT values showed dissimilar dissolution profiles. Dissolution at pH 7.4 with a rotation speed of 100 had

acceptable discrimination ability, but level B and C IVIVCs failed. Hence, for further development, dissolution conditions were chosen to be pH 5.8 and a rotation speed of 100 rpm. The dissolution rate of levosimendan from modified-release formulations is only slightly extended. Most likely the main absorption site is the upper part of the small intestine, and pH 5.8 mimicked *in vivo* dissolution conditions better than pH 7.4.

This study suggests for the first time that the comparison of similarity factors of dissolution profiles vs. differences in pharmacokinetic parameters is useful as a criterion in selection of a dissolution method for level A IVIVC models. Typically, level B and C IVIVC models have been used for selection of dissolution methods (Lake et al., 1999). In our case, level B and C IVIVC alone could lead to selection of an over-discriminating dissolution method. At pH 5.8, both rotation speeds, 50 and 100 rpm, had acceptable (level B or C) IVIVC, but the lower speed was over-discriminating, whereas the higher speed had an acceptable discrimination ability. An over-discriminating dissolution method as a quality control tool could lead to unnecessary rejection of batches during stability studies. It could also lead to failure of such modifications as scale-up of clinical batches, changes to the formulation and changes to the manufacturing method. The development of a level A IVIVC model may also fail if the dissolution method does not have proper discrimination ability. Thus, allocation of resources for dissolution method development in the early drug development phase is justified in order to avoid unnecessary formulation development efforts and *in vivo* studies.

A level A IVIVC model with a Bayesian approach to formulation series (II)

As with dissolution method selection, the construction of an in vitro-in vivo relationship may be challenging. Especially with high variability drugs, level A IVIVC models may fail. This variability may be due to formulation effects and differences in individual subjects. In many cases, the subject related variability is much greater than the variability between formulations (Mauger and Chinchilli, 1997). *In vitro* dissolution data shows only formulation differences. If the drug substance and formulations have high intra- and/or inter-subject variability, many subjects are needed for *in vivo* studies; otherwise, level A IVIVC may not be obtained. In the early phase of drug development, it is difficult to estimate what a sufficient sample size would be, since *in vivo* data of controlled-release formulations is not yet available. Resources and time would be saved, and the necessity

for healthy volunteers would be minimised, if subject-specific *in vivo* data could be utilised effectively in the level A IVIVC model development.

The *in vivo* data of levosimendan modified-release capsules had high variability and only nine subjects were involved in the study. With this data, the averaged level A IVIVC model failed. External prediction errors for C_{max} were 15 and 70% for formulations E and F. The current maximum prediction error allowed according to regulatory guidances is 10% (FDA guidance, 1997, EMEA, 2000). With these challenging data, a stochastic approach was adopted in level A IVIVC model development. A Bayesian approach was integrated with the level A IVIVC model. The *in vitro-in vivo* relationship was described with modified one-compartment model parameters. The Bayesian approach consisted of prior data of model parameters and likelihood function, which defined the connection between *in vitro* and *in vivo* data a with modified one-compartment model. As a result of the simulations, posterior distribution of model parameters, pharmacokinetic parameters and time courses were obtained. Subject specific *in vivo* data was utilised and thus all predictions were found as probability distributions. Posterior distributions represent uncertainty and variability related to the data and the suitability of the modified one-compartment model. The level A Bayesian IVIVC model had good external predictability: the observed mean *in vivo* concentrations were mostly within the predicted 95% posterior probability intervals. Uni-modality and narrow distributions of modified one-compartment model parameters support the reliability of the level A IVIVC model. The constructed model enables prediction of the levosimendan concentration profiles in plasma based on *in vitro* dissolution data alone. The level A IVIVC model with Bayesian approach is a useful tool in the development of levosimendan modified-release formulations.

In general, the Bayesian level A IVIVC models can be utilised for product development purposes if the rate-limiting step for absorption is the dissolution phase. The one-compartment model as a level A IVIVC model with the Bayesian approach requires the same AUC values for all formulations used in the level A IVIVC model development, because differences in AUC can not be predicted with this type of model. AUC values are the same for all formulations when the drug is absorbed to the same extent from all of them. Formulations can have effect on the extent of absorption when they have different absorption windows, e.g. some formulations dissolve already in the small intestine and others have slower dissolution, in which part of the drug is dissolved and absorbed in the

colon. In this case, AUC values for formulations can differ. In our study, the AUC of levosimendan modified-release formulations was not dependent on the formulations.

In our study, a modified one-compartment model with a Bayesian approach was used in direct convolution. Thus, an oral solution, i.v., bolus or immediate-release dosage form is not needed as a reference, as it is in many level A IVIVC models published earlier (Eddington et al., 1998; Modi et al., 2000; Balan et al., 2001; O'Hara et al., 2001; Pitsiu et al., 2001; Sirisuth et al., 2002a). Our study is the first in which a stochastic IVIVC model was developed and validated for a formulation series.

To use the stochastic level A IVIVC model for regulatory purposes, new validity criteria are needed. Current criteria are based on single values, and as such they are intended for averaged IVIVC models (FDA guidance, 1997, EMEA, 2000). To set up validity criteria for stochastic IVIVC models, several different kinds of drugs and products should be studied. To generate a level A IVIVC model with the Bayesian approach, high-level mathematical skills are needed. New user-friendly software would enable extended utilisation of stochastic IVIVC models. More efficient utilisation of *in vivo* bioavailability data and stochastic IVIVC models would save time and costs, and reduce the number of healthy volunteers needed during new drug development. A stochastic level A IVIVC model may be achieved even in cases where averaged IVIVC models have failed.

6.2.2 Simulation studies for proposing biowaver candidates and estimating risks related to bioequivalence (IV)

A dynamic simulation model, in which time dependent drug substance, drug product and gastrointestinal tract physiology-related variables can be easily taken into account, is a useful tool to estimate risks related to test and reference product bioequivalence and to suggest biowaiver candidates. Utilisation of a transparent pharmacokinetic simulation model, which covers a wide and realistic range of biopharmaceutical and pharmacokinetic variables, including elimination, has not been reported previously in the context of bioequivalence and biowaivers. In our simulation studies, an oral solution was compared to the solid dosage form. The effects of absorption and elimination rates on C_{max} and AUC differences were studied, as well as different formulation types and variability in gastric emptying rates.

We considered a 10% difference in AUC and C_{max} as the limit for low risk of failure in bioequivalence studies. In the current bioequivalence guidelines, the acceptable difference is 20% with 90% confidence intervals. The 10% difference in our study represents drug product-related effects i.e. the effect of dissolution rate and formulation type together with intra- and inter-individual variability related to gastric emptying. Variability related to permeability, distribution and elimination phases of drugs, and gastrointestinal tract physiology factors like small intestine transit time or variable pH conditions in the stomach and intestine was not taken into account. However, transit in the small intestine does not show high intra-individual variability and it is not dependent on formulation types or nutrition status (Christensen et al. 1985; Davis et al. 1986; Coupe et al. 1991; Wilding et al. 2003). Variable pH conditions are not critical for high solubility (BCS I and III) drugs.

A dynamic pharmacokinetic model has not been previously used to estimate risks that are related to biowaiver decisions. The basic idea of biowaiver eligible compounds is that the rate and the extent of oral drug absorption should not be dependent on dissolution and/or gastrointestinal transit time (FDA guidance, 2000). Thus, the oral dosage forms should behave like an oral solution and the gastric emptying of the dissolved drug is the rate-limiting step for absorption. Based on that, C_{max} and AUC values for the solid dosage form were compared to values for oral solution. A dissolution rate constant of 2 h^{-1} (>85% dissolved in 60 min) and the current BCS biowaiver requirement for rapid *in vitro* dissolution (4 h^{-1}, >85% dissolved in 30 min) were both studied. It is important to study the slower dissolution rate because *in vivo* dissolution in the stomach is, in many cases, probably slower than *in vitro* dissolution. *In vitro* dissolution tests are carried out with high rotation speeds of basket or paddle (50-100 rpm), whereas in the fasting stomach there are long periods of little or no motor activity (Dressman et al., 1998). A high volume of liquid (500-900 ml) is used for *in vitro* tests as compared to the conditions in stomach, where liquid volume is initially about 250 ml, but decreases rapidly to 50 ml (Wilson and Washington, 1989; Schiller et al., 2005). Based on these physiological facts, we assumed that *in vivo* dissolution is slower than *in vitro* dissolution. Our study is the first to evaluate the influences of formulation types, physiology of the gastrointestinal tract and drug properties including elimination against the current biowaiver criteria.

BCS I drugs

Some of the BCS I drugs were good biowaiver candidates, but a notable portion were not (Fig. 16). Interestingly, C_{max} was highly dependent on both absorption and elimination rates. The combination of rapid absorption and elimination resulted in 10-25% differences in C_{max} values and such compounds were also sensitive to differences in formulation type and gastric emptying rates. These types of BCS I drugs have a high risk of failure in bioequivalency studies and thus *in vivo* studies should be carried out. In contrast, for BCS I drugs with elimination rate constants of < 0.2 h^{-1}, the differences in C_{max} and AUC values with both formulation types and with all gastric emptying rates were less than 5%. These drugs have low risk of failure in bioequivalence studies and they are good candidates for biowaivers. Based on our simulations, the rate of drug elimination should also be taken into account. Follow-up simulations support our conclusions (Fagerholm, 2007). Very high permeability and a short half-life caused difference in C_{max} and AUC when dissolution rate were reduced from 4 h^{-1} to 1 h^{-1}.

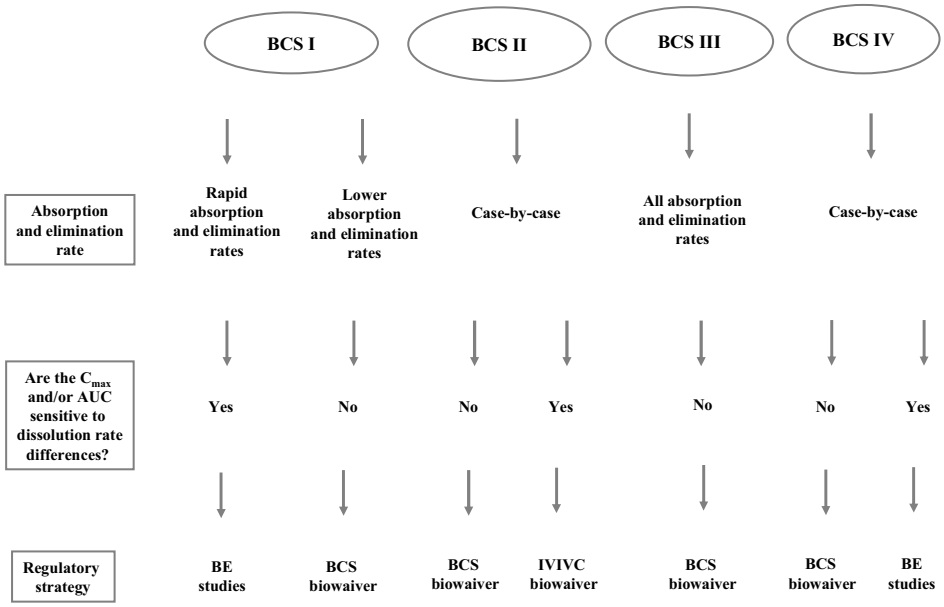


Figure 16 Utilization of dynamic pharmacokinetic modelling in selection of regulatory strategies.

Acidic BCS II drugs

The C_{max} and AUC values of a considerable number of acidic BCS II drugs are dependent on absorption and elimination rates as well as solubility and dissolution (Fig. 16). Thus, the risks related to bioequivalence should be evaluated on a case-by-case basis for this class of drugs, so that drug products which have low risks of failure in bioequivalence studies can be identified. Dissolution is the rate-limiting step for many BCS II drugs. They have a higher risk of failure in bioequivalence studies, but in those cases a level A IVIVC may be found and *in vitro* dissolution may be used as a surrogate for *in vivo* bioequivalency studies.

BCS III drugs

Based on current regulatory guidelines, BCS III drugs are not accepted for biowaivers. However, this class has been proposed in many publications as appropriate for biowaivers if the effects of excipients on the gastrointestinal transit time and permeability can be excluded (Blume, Schug 1999; Yu et al. 2002; Cheng et al. 2004; Vogelpoel et al. 2004; Jantratid et al., 2006). BCS III drugs have low permeability, which, rather than dissolution, is the limiting factor for absorption. This was clearly seen in a bioavailability study of cimetidine immediate-release and controlled-release formulations (Jantratid et al., 2006). Permeability was the rate-limiting step for *in vivo* absorption even when more than 85% of the drug dissolved in 90 minutes. In other studies, three ranitidine immediate-release tablets were found to be bioequivalent when more than 85% was dissolved in 30 minutes; however, dissolution profiles were dissimilar (Piscitelli et al., 1995; Polli, 1997). The BCS III drug metformin had similar absorption from immediate-release and controlled-release formulations (Balan et al., 2001). Controlled-release formulations had quite slow dissolution. More than 85% was dissolved in 180 minutes. Thus even major changes in dissolution rate do not have an effect on C_{max} or AUC. In many publications, BCS III drugs have been suggested for biowaivers if more than 85% of the drug dissolves in 15 minutes (Yu et al., 2002; Polli et al., 2004; WHO, 2006). Based on a few *in vivo* studies of class III drugs and our simulations, this dissolution limit is conservative and, at the least, a limit of more than 85% dissolved in 30 minutes would be sufficient to guarantee bioequivalency. In our simulations, differences in C_{max} and AUC of BCS III drugs were less than 10% when more than 85% was dissolved in 30 or 60 minutes.

However, slower dissolution rates were not studied.

The effects of excipients on absorption and transport mechanism of BCS III drugs should be evaluated carefully before a biowaiver is applied for. Some excipients may open tight junctions and increase absorption of paracellularly transported drugs like ranitidine (Aungst, 2000). One reason for poor absorption can be drug active efflux to the gut lumen, and since some excipients can be substrates or inhibitors of the efflux proteins, they may increase drug absorption. To be a BCS III biowaiver candidate, the main absorption mechanism should be passive diffusion. Thus, excipients affecting efflux and transport proteins are not of concern in the context of BCS III biowaivers.

BCS III drugs were less sensitive to changes in formulation type and gastric emptying rates than BCS I drugs with rapid or rather rapid absorption and elimination. BSC III drugs are also less sensitive to food effects than BCS I drugs (Lennernäs and Abrahamsson, 2005). The ability of food to increase solubilisation does not increase oral absorption of BCS III drugs, as it does with low solubility drugs. Small intestinal transit time is a critical factor for extent of absorption of BCS III drugs. However, food administration does not have an effect on small intestinal transit time (Davis et al. 1986). It can be concluded that BCS III drugs are not sensitive to physiological factors (gastric emptying rates), food effects or minor changes in drug product (formulation type, dissolution).

Acidic BCS IV drugs

Some BCS IV drugs may be biowaiver candidates but a significant part would not be (Fig. 16). The rate limiting step for absorption is a complex combination of permeability, solubility and dissolution properties. Thus, in many cases *in vivo* bioequivalency studies are needed.

Bioequivalence study design

A pharmacokinetic simulation model can be used to estimate risks related to *in vivo* bioequivalency studies. Simulations and predictions can guide bioequivalency study design. Product sensitivity to dissolution rate differences depends on absorption and elimination rates. C_{max} and AUC ratios for test vs. reference product can be simulated and,

based on that, sample size needed for a bioequivalency study can be estimated. BCS I and II drugs with rapid elimination are the most sensitive to dissolution rate differences. Thus, test and reference product should have similar dissolution profiles in the pH range covering the gastrointestinal tract, and perhaps more subjects are needed in bioequivalency studies than for BCS III drugs. For BCS III drugs, dissolution profiles of test and reference do not have to be similar and fewer subjects may be needed for *in vivo* studies than for rapidly eliminated BCS I and II drugs. However, drugs from any BCS class can have high variability in pharmacokinetics. Saturable active transport mechanisms may be involved in drug absorption or metabolism, drug dissolution or solubility of low solubility drugs may be highly dependent on pH; and differences in gastric emptying rates cause variability to the rate and extent of oral absorption. Any of these factors may lead to a larger number of subjects being needed in bioequivalency studies.

7 Conclusions

Pharmacokinetic models were constructed for bioequivalence risk analysis, biowaiver selection and to asses IVIVC properties. These models are potential tools for formulation development and regulatory applications. The specific results and conclusions are:

1. A predictive and discriminative dissolution method was developed for levosimendan modified-release capsules at a level A IVIVC.

2. A predictive stochastic IVIVC modelling method was developed. The model predicted the concentration profiles of a levosimendan modified-release formulation series as probability distributions based on *in vitro* dissolution data and subject-specific *in vivo* data.

3. Ranitidine, a BCS III compound, is proposed as a biowaiver candidate, because differences in its dissolution rate do not cause risks of bioinequivalence.

4. A pharmacokinetic simulation model is a valuable tool to estimate risks of bioinequivalence and to evaluate potential biowaiver candidate drugs.

5. The simulations suggest that the BCS III drugs have a lower risk of bioinequivalence and they are in general more suitable for biowaivers than BCS I drugs. A short half-life of drug elimination and/or a rapid rate of absorption increase the risk of bioinequivalence for BCS I drugs. BCS I drugs were also more sensitive to gastric emptying rate and formulation type differences than BCS III drugs.

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