Part 4

Describing and predicting bioavailability

The aim of Part 4 is to exemplify how bioavailability may be described and predicted. Regulatory authorities have put effort into identifying when bioavailability may be predicted from dissolution studies. Moreover, the US Food and Drug Administration (FDA) has, with support from biopharmaceutical scientists, introduced the Biopharmaceutics Classification System (BCS) in order to classify drug substances according to whether their permeability and/or solubility is rate limiting for bioavailability (FDA Center for Drug Evaluation and Research, 2000).

In Chapter 4.1, dissolution studies are described, focusing on theory and the methods applied to predict bioavailability by dissolutions studies, i.e. *in vitro–in vivo* correlation. In Chapter 4.2, the BCS is described, as well as the Biopharmaceutics Drug Disposition Classification System (BDDCS) which is a further development of the BCS. The BDDCS classifies drug substances based on whether metabolism and transporters play a role in addition to solubility and permeability in determining the bioavailability of (pro)drug substances and candidates (Gupta *et al*., 2006; Benet *et al*., 2008; Custodio *et al*., 2008). Chapter 4.2 also gives an industrial perspective on how the BCS and BDDCS may be used in the global development process of drug candidates in pharmaceutical industry.

Chapter 4.3 introduces and describes how biosimulation studies may be applied to predict the bioavailability of oral (pro)drug substances and candidates. Some commercially available programs for biosimulating oral absorption are discussed. For further reading on biosimulation in drug development a newly published book edited by Bertau *et al*. (2008) may be a more detailed resource.

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4.1

In vitro dissolution

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An important characteristic for a drug substance, to be formulated in a solid oral dosage form, is the rate at which it goes into solution, from a pharmaceutical formulation from the pure drug substance. This is called the dissolution rate and is relevant since generally only a dissolved drug substance will be absorbed in the gastrointestinal (GI) tract.

Dissolution rates are determined by standardised dissolutions tests, described in the *European Pharmacopeia* (Ph Eur) and the *US Pharmacopeia* (USP), and also in pharmacopoeias covering other countries. The term 'dissolution' is used for all oral dosage forms, such as formulations that aim at a fast release of drug substance (immediate-release or conventional formulations) as well as those with a modified-release profile (controlled, delayed, extended, prolonged or sustained-release formulations). The term 'intrinsic dissolution' covers the dissolution of pure drug substance under controlled conditions.

In general, dissolution testing of pharmaceutical formulations is carried out with one of two purposes in mind: to support selection made during the development of new pharmaceutical formulations, or to perform quality control during production of pharmaceutical products.

When developing formulations for a specific drug substance, a desired dissolution profile is often defined and the development process will aim at reaching a delivery system with this profile. This work often takes place using compendial buffers as dissolution media. More sophisticated dissolution media, simulating the GI fluids, can be used in order to elucidate the potential influence of GI conditions (e.g. food effects) or to obtain a closer correlation with the *in vivo* situation. Development of new pharmaceutical formulation technologies takes place both in the industry and in academia, using *in vitro* dissolution testing as a central part.

For oral drug products on the market, a dissolution test assuring the quality of the product will often be included in the registration material. This dissolution test has been shown to be discriminative for the specific product during the development period and is easy to perform and

reproduce, thus batch-to-batch quality control dissolution tests do not normally include complex media simulating the intestinal fluids. According to the USP, selection of appropriate conditions for routine quality testing should, where possible, be based on discriminatory capability, robustness, stability of the drug substance in the dissolution medium, and the relevance to *in vivo* performance.

As described above, *in vitro* dissolution tests serve as a tool for assessing the biopharmaceutical dissolution properties of a pharmaceutical solid oral dosage form, during its development phase, as well as a quality control of the final marketed product. Furthermore, *in vitro* dissolution data are central for evaluating whether *in vivo* bioavailability studies are needed when changes are made to a marketed product regarding production site, manufacturing process or formulation (International Pharmaceutical Federation (FIP), 1997). If a dissolution method has been proven to be predictive of *in vivo* behaviour, the dissolution test might reduce or even obviate the necessity of performing expensive human bioequivalence studies. These circumstances will be expanded on in Chapter 4.2 by Dr Wu. However, the relationship between *in vitro* dissolution and *in vivo* bioavailability/absorption is still far from being fully explored, and it is important to keep in mind the limitations of the dissolution test when it is used as a predictor of *in vivo* performance.

During the past two decades, the focus has been on developing and optimising dissolution methods in both scientific, as well as regulatory communities. *In vitro* dissolution as a tool in biopharmaceutical evaluations has attracted a lot of interest; dissolution test methodology has been incorporated in the pharmacopoeias, and a large number of regulations and guidelines have been issued on bioavailability, bioequivalence and *in vitro* dissolution testing at both national and international levels (FIP, 1997).

In the present chapter, dissolution will be described and discussed with regard to dissolution mechanism theories, factors influencing dissolution – *in vitro* and *in vivo* – dissolution equipment described in the pharmacopoeias, and selection of dissolution media for *in vitro* dissolution studies.

4.1.1 Dissolution mechanism theories

Dissolution is basically the process molecules undergo when they are transferred from the solid to the dissolved state. There are two steps involved in dissolution. The first is detachment of molecules from the solid surface to form hydrated molecules at the solid–liquid interface. The

second is the transport from this interface to the bulk solution. Depending on which step is rate limiting to mass transfer, the dissolution process can be either:

- reaction-rate controlled
- transport-rate controlled
- a function of both processes.

Reaction rate-controlled dissolution is controlled by the rate of detachment of the drug from its crystal lattice into the solvent. In this case the liberation and deposition of the solubilised molecule is slower than the following process of transport into bulk solution. *Transport rate-controlled* dissolution involves two fundamental processes of mass transfer: molecular diffusion and forced convection. The rate of drug transport away from the dissolving solid surface is a combination of diffusion in the direction perpendicular to the planar surface and convection in the direction of flow.

When the rate constants of both processes are approximately equivalent, the dissolution rate is *a function of both processes*, i.e. determined by both the rate of reaction at the interface and the rate of the transport process (Wurster and Taylor, 1965; Abdou, 1989).

The most common dissolution theory is called the film theory or the diffusion layer model (Abdou, 1989). This theory assumes that the dissolution rate is *transport-rate controlled*, and in fact most dissolution processes are controlled by this diffusion–convection-controlled step (Wang and Flanagan, 1999). A basic diffusion–convection-controlled model for solid dissolution was developed by Noyes and Whitney in 1897, and later modified by Nernst (1904) and Brunner (1904), Levich (1962), and Dressman *et al.* (1998), leading to the equation shown in Equation 4.1.1:

$$\frac{dX_d}{dt} = \frac{A\,D}{\delta}\left(C_s \frac{X_d}{V}\right) \tag{4.1.1}$$

where dX_d/dt is the dissolution rate as a function of the surface area, A, available for dissolution, the saturation solubility, C_S, of the drug substance in the dissolution medium, the amount of drug already in solution, X_d, the diffusion coefficient of the drug substance, D, the volume, V, of dissolution medium, and the diffusion layer thickness, δ, adjacent to the dissolving surface. It is assumed that there is a rate-limiting diffusion layer at the solid–liquid interface, which has been described in the literature as a thin stagnant layer of saturated solution (Wurster and Taylor, 1965) or as

a hydrodynamic boundary layer with a velocity and a concentration gradient (Levich, 1962).

The Noyes–Whitney equation (Equation 4.1.1) is exact for dissolution from a plane surface under sink conditions, because the concentration gradient in the diffusion layer is linear at steady-state (Wang and Flanagan, 1999). Sink conditions are present when the concentration of the drug substance in the bulk phase is low and does not have an influence on the dissolution process from the solid phase. This is normally considered to be in the region of 10–30% of the saturation solubility or lower.

For spherical particles, the Noyes–Whitney equation will not be accurate, because the concentration gradient in the diffusion layer is non-linear. The degree of non-linearity depends on the ratio of drug particle size to diffusion layer thickness (Wang and Flanagan, 1999). Even though the thickness of the diffusion layer can only be calculated accurately if the hydrodynamics in the system are well defined, there are several suggestions in the literature on how to calculate the thickness of the diffusion layer of a particle (Abdou, 1989). Furthermore, it is important to keep in mind that the dissolution of particles will be influenced by the particle shape and particle size distribution.

4.1.2 Factors influencing dissolution *in vitro*

In vitro dissolution of a drug substance from either the pure solid drug or a pharmaceutical formulation is influenced by several factors. These factors can be related to the physicochemical characteristics of the drug substance, the pharmaceutical formulation and the conditions of the *in vitro* dissolution test.

First of all, the solubility of the drug substance in the dissolution media should be known. This will reveal if there will be problems maintaining sink conditions during the dissolution test. This is the case for many poorly soluble drug substances belonging to class 2 or 4 in the Biopharmaceutics Classification System (BCS) (see Chapter 4.2 for further details). If sink conditions cannot be achieved, several possibilities to solve this issue are available: in general the dose of drug can be reduced or the composition of the medium can be changed in several ways. Firstly, the pH of the medium can be changed, taking into account the acid/base properties of the drug substance; the dissolution can be carried out at a pH value where the solubility is higher, thus enabling sink conditions. The pH of the dissolution medium should only be chosen within a certain interval that is physiologically relevant (see later); thus a pH change is generally only

relevant for drugs with pK_a values in the physiological pH range. Acid/base properties of drug substances are further described in Chapter 2.1.

If changing the pH is not feasible, the drug solubility in the media can be changed by increasing the dissolution volume or by addition of surfactants. The solubility of most poorly soluble drugs is enhanced in the presence of surfactants. Surfactants often used for dissolution includes sodium lauryl sulphate (SLS) and polysorbate 60 or 80 (Tween 60 or 80) as well as various sorbitan esters (SPANs). In some rare instances, the use of ethanol to increase dissolution has also been reported.

Dissolution testing is carried out on pharmaceutical formulations, and the characteristics of the formulation have to be taken into consideration. Several approaches can be applied when formulating a poorly soluble drug, where the solubility and dissolution rate in the GI tract are often the limiting factors for the absorption.

Firstly the dissolution rate of a drug substance can be increased by decreasing its particle size and thereby increasing the surface area available for dissolution. Furthermore, surfactants can be added to the formulations in order to give a better wetting of the drug substance and possibly increase the solubility. For drug substances that exhibit polymorphism, i.e. where the compound can exist in two or more crystal structures, the choice of crystal structure will influence the dissolution rate if there is a difference in the solubility of each polymorphic form.

Another strategy that can be used for poorly soluble drugs is to stabilise the drug substance in an amorphous state by using polymers (Chokshi *et al.*, 2007; Thybo *et al.*, 2008). The high-energy amorphous state has a higher dissolution rate than the crystalline form and this might result in an increased bioavailability. The amorphous state is normally physically unstable, so if it has not been stabilised in some way there might be a transformation into a crystalline phase over time.

The important parameters of the dissolution test include the volume and composition of the dissolution medium as well as the hydrodynamics employed and the duration of the test. These parameters influence the dissolution by exerting effects on solubility, effective surface area or diffusivity of the drug substance (Dressman *et al.*, 1998; Nicolaides *et al.*, 2001). The test conditions normally employed, and alternative suggestions, will be presented in a later section of this chapter.

4.1.3 Factors influencing dissolution *in vivo*

When considering the *in vivo* dissolution rate of a drug substance, this will be influenced by the interplay with the physiological conditions in the

Table 4.1.1 Physicochemical and physiological parameters important to drug dissolution in the gastrointestinal tract; modified from Dressman *et al.* (1998)

Factor	*Physicochemical parameter*	*Physiological parameter*
Surface area of drug (S)	Particle size, wettability	Surfactants in gastric juice and bile
Diffusivity of drug (D)	Molecular size	Viscosity of luminal contents, diffusivity of mixed micelles
Boundary layer thickness (δ)		Motility pattern, flow rate
Solubility (C_s)	Hydrophobicity, crystal structure, pK_a	pH, buffer capacity, bile, food
Amount of drug already dissolved (X_d)	Particle size, wettability, solubility	Permeability
Volume of solvent available (V)		Secretions, co-administered fluids

GI tract and the physicochemical properties of the drug substance as well as by the properties of the pharmaceutical formulation. In Table 4.1.1 these factors are summarised according to the parameters from the Noyes–Whitney equation.

The aim of investigating *in vitro* dissolution for an oral solid dosage form is, in the present context, to obtain an estimate of its *in vivo* dissolution. This is a difficult task, considering the very complex nature of the GI tract. Reference is made to Chapter 3.1 where the GI tract is described. The present section will focus on some of the characteristics of the GI tract that are important to the dissolution process. The *in vivo* dissolution may be influenced by volume, pH and composition of the GI fluids. Furthermore, the hydrodynamics and the transit times in the different parts of the GI tract may have an influence on the *in vivo* dissolution rate.

The volume of the fasted stomach can be as low as 20–50 ml (Davenport, 1977; Dressman *et al.*, 1998) but changes when a drug is administered together with fluid. In the fed state, the volume is dependent on the composition and the volume of the meal. The capacity of the human stomach is approximately 1–1.6 l (Kararli, 1995). The volume of the fasted small intestine has been found to be 120–350 ml (Dillard *et al.*, 1965), while Fordtran and Lochlear (1966) reported volumes in the upper small intestine in the fed state of up to 1.6 l. There is a large fluid flow in the intestines, with an average of 9 l being presented to the

intestines per day. Approximately 2 l originate from oral ingestion and 7 l from endogenous secretions (Chang and Rao, 1994). The fluid volume is lower in the distal parts of the intestines, where approximately 1.5 l enters the colon daily and about 1.3 l is absorbed during the passage of the chyme in the colon.

The pH of gastric fluid in the fasting stomach is between 1.5 and 2.9 (Dressman *et al.*, 1990; Lindahl *et al.*, 1997, Kalantzi *et al.*, 2006), while the pH of the fed stomach is dependent on the composition of the meal; pH values up to 5 have been reported in the stomach contents after intake of a meal (Dressman *et al.*, 1990). In the fasted duodenum and upper jejunum, average pH values between 6 and 7.1 have been observed (Dressman *et al.*, 1990; Lindahl *et al.*, 1997; Fallingborg, 1989; Kalantzi *et al.*, 2006). Food intake results in a slight reduction in the upper intestinal pH; values in the range 5.5 to 6.5 have been reported (Fallingborg *et al.*, 1989; Persson *et al.*, 2005; Kalantzi *et al.*, 2006).

The surface tension in the GI fluids is much lower than that of water, which is 72 mN m^{-1}. Values between 30 and 50 mN m^{-1} have been reported in gastric fluids from fasted subjects (Finholt and Solvang, 1968; Efentakis and Dressman, 1998; Pedersen *et al.*, 2000; Kalantzi *et al.*, 2006). In the fasted small intestine, the surface tension has been measured to be between 30 and 34 mN m^{-1} (Pedersen *et al.*, 2000; Kalantzi *et al.*, 2006). In the fed state, the surface tension in the stomach is dependent on the composition of the meal, while it does not change in the upper small intestines between the fasted and fed state (Persson *et al.*, 2005; Kalantzi *et al.*, 2006).

Besides lowering the surface tension of the GI fluids, the presence of endogenous surfactants in the GI tract can be expected to increase the solubility of poorly soluble drug substances as well as giving a better wetting of solid particles compared to water. The main endogenous surfactants in the small intestines are bile salts and phospholipids from bile. After food intake, several surfactants from the meal may be present, e.g. proteins and dietary phospholipid. Digestion of food can also produce surfactants, such as fatty acids and monoglycerides generated by lipolysis of triacylglycerides from fats. Furthermore, it is also important to consider the presence of salts, impacting the ionic strength in the GI fluids. Then there is the issue of a possible interaction between a drug substance and the ingested food, which should be taken into account when developing an oral pharmaceutical formulation.

The residence times or transit times through the different compartments in the GI tract are shown in Table 3.1.2 in Chapter 3.1. It is

important to take these into account when deciding how long to conduct the dissolution test for. The data in Table 3.1.2 are average values and it might be important to consider the range of residence times in some cases. Absorption of drug substance occurs primarily in the intestine, so the gastric emptying of fluid or particle matter is one rate-limiting factor for the absorption process. In case of the fasted state, the gastric emptying is dependent on the motility phase present when the drug is ingested. In the fasted state there is a cyclic motility pattern present in the stomach and intestines. This pattern is described by three, sometimes four phases (Oberle and Amidon, 1987; Sarna, 1985). Phase I is the quiescent period with no activity. Phase II consists of intermittent and irregular contractions which gradually increase to a short period of intense contractions called phase III. Phase III is also called the housekeeper wave or the interdigestive migrating motor complex (MMC). Phase IV is the short transition period between phases III and I. The gastric emptying of aqueous solutions in fasted human subjects for 50 ml and 200 ml has been shown to give a $t_{50\%}$ between 5 and 61 min, depending on which phase was present when the solution was ingested (Oberle *et al.*, 1990). The gastric emptying of non-disintegrating tablets, e.g. modified-release dosage forms, in the fasted state typically follows the MMC phase III, the housekeeper wave. This results in a large variation in the gastric emptying time within the 2 h that the MMC cycle usually lasts (Podczeck *et al.*, 2007a,b). The presence of food in the stomach has been shown to delay the emptying of larger single units such as tablets and capsules, while emptying of small pellets was not greatly affected by the fed state (Davis *et al.*, 1986). Gastric residence times from a few minutes to many hours have been reported in the literature (Fallingborg *et al.*, 1989; Davis *et al.*, 1986; Podczeck *et al.*, 2007c).

The intestinal transit times of pharmaceutical dosage forms including data from both the fasted and the fed state have been measured to be between 2 and 5 hours (Davis *et al.*, 1986). These data included the small intestinal transit time of solutions, pellets and large single-unit dosage forms.

In vivo the drug substance disappears after release from an oral dosage form, due to absorption to the systemic circulation. Therefore, it is often assumed that sink conditions are present *in vivo*. For simulations of dissolution in the stomach, sink conditions do not represent a problem, since absorption across the gastric mucosa is usually negligible. However, for highly permeable drugs with fast absorption, sink conditions might be maintained in the small intestine.

4.1.4 Dissolution equipment described in the pharmacopoeias

The Ph Eur and the USP describe four different types of dissolution equipment for oral dosage forms: the basket (Apparatus 1, Figure 4.1.1) and the paddle (Apparatus 2, Figure 4.1.2), both appearing in the pharmacopeias in the 1970s, and the reciprocating cylinder (Bio-Dis, apparatus 3, Figure 4.1.3) and the flow-through apparatus (apparatus 4, Figure 4.1.4) that were included in 1990s. One further piece of equipment for the determination of the intrinsic dissolution of a pure drug substance is described in the pharmacopoeias.

A number of other pieces of specialised dissolution apparatus have been proposed over the years, and the reader is referred to Shiu (1996) for further details. Furthermore, guidelines for dissolution testing of novel or special dosage forms from the FIP and American Association of Pharmaceutical Scientists (AAPS) has been published. These include dosage forms such as suspensions, chewable tablets, transdermal patches and implants (Siewert *et al.*, 2003).

The basket, paddle, flow-through apparatus and intrinsic dissolution will be described in more detail in the following sections. The reciprocating cylinder apparatus can be applied to material that is contained in the glass reciprocating cylinder and does not go through the mesh screen (see Figure 4.1.3). The reciprocating cylinder is advantageous over the closed systems (see later) in that the dosage form can move about freely and does not come into contact with the walls (Esbelin *et al.*, 1991). In the commercially available apparatus there are several glass vessels for each reciprocating cylinder and this makes it possible to change the dissolution medium several times in one test.

4.1.4.1 The paddle and basket apparatus

The most commonly used pieces of dissolution equipment are the basket and the paddle apparatuses. They can be characterised as 'stirred beaker' methods and are simple, robust, well standardised and easy to use. They are also referred to as 'closed systems' because they use a fixed volume of dissolution medium (Shiu, 1996; Dressman, 2000). The basket method is generally preferred for capsules, whereas tablet dissolution is performed using the paddle method. If the paddle method is employed for capsules, sinkers can be used to keep the capsules from floating. Sinkers are made of non-reactive material, and several designs and sizes are available on the market.

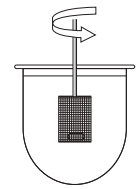

Figure 4.1.1 Dissolution apparatus 1 (basket).

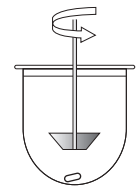

Figure 4.1.2 Dissolution apparatus 2 (paddle).

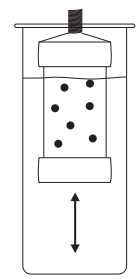

Figure 4.1.3 Dissolution apparatus 3 (reciprocating cylinder).

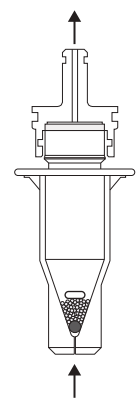

Figure 4.1.4 Dissolution apparatus 4 (flow through).

For the basket and paddle apparatus, vessels of 1, 2 or 4 l can be employed. The 1 l vessel is the most commonly used, with typical volumes of 500, 900 or 100 ml dissolution medium. Dressman *et al.* (1998) suggest a medium volume of 300 ml to simulate the fasted stomach, 500 ml for the fasted small intestine, and up to 1000 ml for fed-state conditions in the stomach and small intestine. The USP suggests the use of medium volumes up to 2 l for poorly soluble drug substances. The quantity of the medium should be at least three times the amount required to form a saturated solution of the compound in order to obtain sink conditions.

In general, mild agitation is to be used during dissolution testing to allow maximum discriminating power. The FDA recommends rotating speeds of 50–100 rpm for the basket method and 50–75 rpm for the paddle method for testing solid oral dosage forms (FDA, Center for Drug Evaluation and Research, 1997). The Ph Eur (2.9.3) suggests the use of speeds between 50 and 100 rpm for both methods and that the speed must not exceed 150 rpm.

4.1.4.2 Flow-through dissolution apparatus

In the flow-through apparatus, the dosage form is placed in a small vertical cylinder flow cell that is flushed continuously in an upward direction with a stream of dissolution medium (see Figure 4.1.4). The bottom cone is usually filled with 1 mm glass beads with one glass bead of 5 mm at the apex to protect the fluid entry tube. A laminar flow is secured by placing these small glass beads in the bottom of the flow cell (Zhang *et al.*, 1994). In the inner top of the cell, a glass-fibre filter can be placed. The dosage form can be placed on top of the glass beads as recommended in the pharmacopoeias, but can also be embedded in the glass beads. The placement of the dosage form will impact on the dissolution rate. Besides the placement of the dosage form, the main parameters determining the dissolution in the flow-through dissolution cell are the flow rate, the dissolution time and the composition of the dissolution media.

There are two sizes of flow cell described in the pharmacopoeias; one large cell with a diameter of 22.6 mm (shown in Figure 4.1.4) and one small cell with a diameter of 12 mm. The flow rates suggested in Ph Eur and USP are 4, 8 and 16 ml min^{-1}. The intestinal fluids' axial velocity has been estimated to be in the region of 1.5 cm min^{-1}, while the fluid flow inside the 22.6 mm cell is 1, 2 and 4 cm min^{-1} for the dissolution medium flow rates of 4, 8 and 16 ml min^{-1}, respectively (Fotaki *et al.*, 2005). The flow pattern in the flow-through dissolution equipment is unidirectional and probably unrepresentative of the segmental mixing in the intestine.

In vitro–in vivo correlations may be obtained by performing the dissolution test at a higher flow rate to compensate for the lack of destructive hydrodynamics. The *in vitro–in vivo* correlation between different flow rates as well as different dissolution media employed in the flow-through apparatus has been discussed by Sunesen *et al.* (2005).

One advantage is that the flow-through apparatus offers the possibility of maintaining sink conditions by continuous removal of dissolved drug (Zhang *et al.*, 1994; Perng *et al.*, 2003). However, the flow-through method has not been routinely used for immediate-release products, mainly because it is laborious and expensive due to the need for large volumes of medium. It is mainly used in cases where the performance of the paddle and basket apparatus is unsatisfactory. For several formulations though, the flow-through apparatus has been found to be superior to the paddle apparatus in achieving *in vitro–in vivo* correlation for several formulations (Ammar and Khalil, 1993; Butler and Bateman, 1998; Bloomfield and Butler, 2000).

4.1.4.3 Intrinsic dissolution

The intrinsic dissolution rate is often measured on the pure drug substance in the early development phase during the development of new drugs in the industry. According to the USP:

> The measurement of intrinsic dissolution rates is a tool in the functionality and characterisation of bulk drug substances and excipients. The intrinsic dissolution rate is defined as the dissolution rate of pure substance under the condition of constant surface area (USP 28, 1087).

When testing the intrinsic dissolution rate, the substance is compacted at high compression force to obtain a disk with a smooth surface area. This is normally done without adding any excipient such as lubricants or binders. The disk is preferably compressed in the disk holder but can also be compressed and then placed in a disk holder.

When studying different polymorphs of a substance, it might be necessary before measuring the dissolution rate to check if the high compression force imposes a change in the crystal structure.

The advantage of the intrinsic dissolution apparatus is that the area of drug is kept constant during dissolution and that the hydrodynamics are well defined. Equation 4.1.2, developed by Levich in 1962, can be used for calculations of intrinsic dissolution rate results:

$$J = 0.62D^{2/3}\nu^{-1/6}\omega^{1/2}\,C_s \qquad (4.1.2)$$

where J is the flux, D is the diffusion coefficient, ν is the kinematic viscosity, ω is the rotational speed and C_s is the saturation solubility in the dissolution medium. Yu *et al.* (2004) have suggested that measurements of the intrinsic dissolution rates can be used for classifying drug substances into the BCS as low- or high-solubility drug substances. They measured the dissolution rate of 15 model drug substances and found that a dissolution rate of 0.1 mg min^{-1} cm^{-2} was the class boundary unless the dose of the drug substance was extremely low or high (Yu *et al.*, 2004).

4.1.5 Selection of dissolution media for *in vitro* dissolution studies

> The testing conditions should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration (FDA, Center for Drug Evaluation and Research, 1997).

Very often the *in vitro* dissolution is more sensitive to variations than the *in vivo* performance. Thus, to achieve predictive *in vitro* data, it is important to identify and incorporate the rate-limiting physiological variable for *in vivo* dissolution (Shah and Lesko, 1995).

4.1.5.1 Compendial dissolution media

According to the USP, the pH of a dissolution medium for an oral dosage form should be in the physiological range of 1.2 to 6.8. For modified-release dosage forms, pH values up to 7.5 could be evaluated, due to the potential release for the drug substance in the lower regions of the intestine. For certain drug substances it can also be of relevance to measure the pH change in the medium during the dissolution test.

Several buffer solutions are suggested in the Ph Eur and USP to perform dissolution at different pHs. In Table 4.1.2 the examples of dissolution media from the Ph Eur are shown. Reference is made to the pharmacopoeias for details on how to prepare these dissolution media.

The pharmacopeias also recommend the use of media that are closer to the *in vivo* fluids, e.g. simulated (or artificial) gastric juice containing pepsin in addition to HCl and NaCl. Inclusion of pepsin in the dissolution medium might be appropriate when working with gelatine capsules. The media recommended by the Ph Eur as 'simulated intestinal fluid' contain a phosphate buffer (pH 6.8) and pancreas powder. However, it should be noted that these media do not have the lower surface tension that

Table 4.1.2 Examples of dissolution media from Ph Eur's chapter on 'Dissolution test for solid dosage forms' monograph 2.9.3

pH	*Dissolution media*
1.0	0.2 M HCl
1.2	85 mM HCl, 50 mM NaCl
1.5	41.4 mM HCl, 50 mM NaCl
4.5	0.1 M phosphate or 22 mM sodium acetate and 28 mM acetic acid = acetate buffer
5.5 and 5.8	0.1 M phosphate buffer or 44 mM sodium acetate and 6 mM acetic acid = acetate buffer
6.8	50 mM phosphate buffer
7.2 and 7.5	50 mM phosphate buffer

characterises the human GI fluids; furthermore, the activity of the added enzymes is not controlled.

In order to simulate transfer from the stomach to the intestine, a change of media during dissolution is also recommended by the pharmacopeias. This is especially relevant for enteric-coated or delayed-release dosage forms. The pharmacopoeias recommend two different methods for changing the pH during a dissolution test, both meant for the paddle and basket methods. 'Method A' is performed in one vessel, while 'method B' includes transfer of the dosage form from a vessel containing an acidic medium simulating the gastric fluid, to a vessel containing a medium buffered at pH 6.8. For 'method A', the first part of the study is performed in 750 ml 0.1 M HCl. After 2 h, 250 ml of 0.2 M phosphate buffer is added and the pH adjusted to 6.8. The test continues for 45 min or for the specified time. The dissolution medium for 'method B' prescribes 1 l 0.1 M HCl for 2 h and 1 l phosphate buffer, pH 6.8 for 45 min, or for the specified time.

4.1.5.2 Biorelevant dissolution media

Media simulating the gastric and intestinal fluids to a much higher degree than the compendial media described above have also been developed; these are the so-called biorelevant media.

As already described, fasted-state gastric fluids are characterised by a lower surface tension than water, and a pH between 1.5 and 2.9, which is higher than the pH of compendial gastric media. Vertzoni *et al.* (2005) proposed a fasted-state gastric medium (fasted-state simulated gastric

Table 4.1.3 Examples of biorelevant dissolution media. FaSSGF: fasted-state simulated gastric fluid; FeSSGF: fed-state simulated gstric fluid; FaSSIF: fasted-state simulated intestinal fluid; FeSSIF: fed-state simulated intestinal fluid

	FaSSGF	*FeSSGF*	*FaSSIF*	*FaSSIF–v2*	*FeSSIF*	*FeSSIF–V2*
Milk:buffer	–	1:1	–	–	–	–
Sodium taurocholate (mM)	0.08	–	3	3	15	10
Lecithin (mM)	0.02	–	0.75	0.2	3.75	2
Glycerol mono-oleate (mM)	–	–	–	–	–	5
Sodium oleate (mM)	–	–	–	–	–	0.8
Pepsin (mg ml^{-1})	0.1	–	–	–	–	–
Sodium chloride (mM)	34.2	237.02	106	68.62	173	125.5
Acetic acid (mM)	–	17.12	–	–	144	–
Sodium acetate (mM)	–	29.75	–	–	–	–
Sodium dihydroxy phosphate (mM)	–	28.66	–	–	–	–
Sodium hydroxide (mM)	–	–	–	34.8	–	81.65
Maleic acid (mM)	–	–	–	19.12	–	55.02
pH	1.6	5	6.5	6.5	5	5.8
Osmolality (mOsmol l^{-1})	120.7 ± 2.5	400	270 ± 10	180 ± 10	63 ± 10	390 ± 10
Buffer capacity	–	25	12	10	76	25

fluid; FaSSGF) with a pH of 1.6 and a reduced surface tension mediated by pepsin and low levels of taurocholate and phospholipids (see Table 4.1.3). FaSSGF was shown to give an *in vitro* dissolution profile of several drug models that predicted the *in vivo* dissolution in a satisfactory way.

Simulating the post-prandial stomach is complex, since the fed-state gastric fluids will be very dependent on the food ingested. Milk and nutritional drinks have been suggested as fed-state gastric media (Macheras *et al*., 1987; Galia *et al*., 1998; Klein *et al*., 2004), used both pure and diluted with different buffers. Recently Jantratid *et al*. (2008) proposed the use of three media simulating the content of the fed stomach over time after meal intake. In order to simulate early digestion, pure milk adjusted to pH 6.5 was used; later digestion stages were simulated by milk:buffer 1:1 at pH 5, while milk:buffer 1:3 at pH 3 was used to

simulate late phases in digestion. The buffer capacity of the media and osmolarity were also taken into account. However, the final proof of the adequacy of any of these media to simulate the actual *in vivo* dissolution of a given compound is yet to be demonstrated.

Many different media simulating the intestinal fluids have also been recommended. In 1998 Dressman and co-workers (Galia *et al.*, 1998) proposed a fasted-state simulated intestinal fluid (FaSSIF) and a fed-state simulated intestinal fluid (FeSSIF), the two media diverging by the level of taurocholate, the osmolality and the pH (see Table 4.1.3). These media have since been widely used in both industry and academia to characterise drugs and formulations. However, the FeSSIF media do not contain surface-active compounds like fatty acids and monoglycerides generated from digestion of lipids and other food components in the intestine. Other media containing lipid-digestion products have been included in biorelevant media used for solubility studies (Grove *et al.*, 2005; Nielsen *et al.*, 2005). Lipid-digestion products have been shown to be very important in simulating *in vivo* drug dissolution in the fed state (Sunesen *et al.*, 2005; Lue *et al.*, 2008). In these two publications, it is found that *in vivo–in vitro* correlation in the fed state can only be achieved by the use of biorelevant dissolution media containing lipid-digestion products. Recently, a modification of media simulating the upper small intestinal fluids was suggested (Jantratid *et al.*, 2008). Here the authors have included lipid-digestion products, and also simulate different stages of digestion, with different level of surfactants. However, the final proofs of the *in vivo* relevance of these media are still awaited.

4.1.6 Conclusions

Standardised dissolution tests are described in the pharmacopeias and are used for quality control for most oral dosage forms. During development of an oral pharmaceutical formulation, dissolution tests are used on several levels: to determine the intrinsic dissolution rate of the drug substance, and to characterise the drug release from the formulations in the development phase. Usually a specific dissolution profile, employing a specific dissolution test, is desired and the development work aims to achieve this profile.

It is always relevant to consider the correlation with the *in vivo* situation, since formulation optimisation only makes sense if the *in vitro* dissolution profile reflects the *in vivo* conditions. Here it should be considered that the dissolution test apparatuses and media described in the pharmacopeias are very far from the *in vivo* situation. The *in vivo*

dissolution process is very complicated and the geometry and hydrodynamics prevailing in the *in vitro* dissolution test are very different from these. However, several publications have reported on achieving *in vitro–in vivo* correlation, so by using well-selected *in vitro* dissolution conditions, it seems to be possible to simulate the *in vivo* situation.

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4.2

The Biopharmaceutics Classification System in drug discovery and development

Chi-Yuan Wu

There is both economic interest and medical need for exploring and improving the oral bioavailability of drugs in development. During the drug discovery process, drug candidates are screened for their 'absorptive, distributive, metabolic and eliminative' (ADME) properties. This is because ADME properties, which may also be called 'drug metabolism and pharmacokinetic' (DMPK) properties, are descriptive for evaluating the potential of the candidate to become a new drug product. Nowadays, DMPK issues are evaluated earlier in the discovery process than in former times and that is one of the reasons why the pharmaceutical industry has a higher success rate in introducing new drug substances into the market. The value of systematic approaches to predicting pharmacokinetic profiles is better appreciated, as this can drastically reduce the time and expense of drug development. Since pharmacokinetics are predicted from a limited set of input data, in the screening process a variety of algorithms have been proposed to evaluate the correlation between pharmacokinetic properties and pharmacological activities. For instance, methods to modify dissolution or aqueous solubility, decrease degradation of the drug candidate in gastric and intestinal fluids, enhance poor intestinal membrane permeation, and inhibit pre-systemic intestinal or hepatic metabolism have been proposed and studied.

Traditionally, the most pharmacologically active candidates were advanced to the next stage of development. Unfortunately, a significant percentage failed as a result of poor pharmacokinetic properties. An emerging strategy is to optimise both pharmacological activity and pharmacokinetic properties during drug discovery (see Figure 4.2.1). Bioavailability can be improved by modifying membrane permeability. Compounds with poor intestinal permeability have been associated with certain physicochemical properties, such as: low octanol/aqueous partitioning, the presence of strongly charged functional groups, high molecular

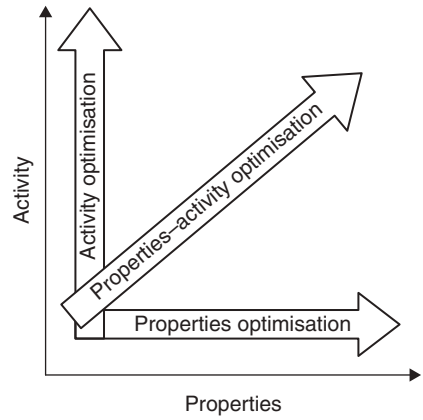

Figure 4.2.1 Schematic representation of the pharmaceutical profiling optimisation.

weight, and a substantial number of hydrogen-bonding functional groups (Lipinski *et al*., 1997). Lipinski's rule of five is further described in Section 2.4.1. Candidates that may benefit most from intestinal absorption-enhancing formulations usually have one or more of the above-mentioned characteristics. Typically, the drug candidates for which absorption-enhancement studies have been involved in their development, have been peptides, peptide analogues, or other polar, high molecular weight drug substances, such as heparin. For some candidates, their permeation through the intestinal epithelium is hindered by active efflux transporters that efflux or exsorb the candidates from the enterocyte back into the intestinal lumen, and/or by the candidate being degraded via drug-metabolising enzymes located in the enterocytes. The exsorptive transporters involved may include P-glycoprotein (P-gp), the family of multidrug-resistance-associated proteins (MRPs), and others. Exsorptive/efflux transporters are further described in Chapter 3.6. The main metabolising enzyme in enterocytes is cytochrome P450 3A (CYP3A). It has only recently been realised that besides physicochemical properties, these biochemical barriers (i.e. enzymes and transporters) also play an important role in controlling drug absorption.

In this chapter we will discuss and emphasise the importance of the fundamentals of the Biopharmaceutics Classification System (BCS). These fundamentals are to determine the solubility and intestinal permeability properties of candidates intended for oral absorption. However, the chapter will also discuss and emphasise how enzyme–transporter interplay can be incorporated into the BCS as a method for predicting drug disposition. The primary use of the BCS is, from a regulatory

perspective, for bioequivalence testing, i.e. to identify drugs for which *in vitro* dissolution testing could replace *in vivo* studies. However, if the BCS is useful for other reasons than regulatory issues, such as to predict drug disposition, its impact would certainly increase. Thus, we emphasise the innovative concept of the Biopharmaceutics Drug Disposition Classification System (BDDCS) implemented as a simple tool in early drug development to identify and determine the major factors for predicting the drug disposition process.

4.2.1 Prediction of oral drug absorption

4.2.1.1 The drug absorption process

The oral absorption of a drug candidate depends on the amount dissolving in the aqueous solution of the gastrointestinal tract. The term 'oral bioavailability' is defined by the European Medicines Agency (EMEA) as the rate and extent to which the drug substance is absorbed from a pharmaceutical formulation and becomes available at the site of action (EMEA, 2001). The term 'absolute bioavailability' is used by the American Food and Drug Administration (FDA) and many others as being equivalent to the term 'absorption fraction' (f_{oral}). f_{oral} may be estimated from the dose-corrected ratio of the area under the blood concentration curve following oral administration (AUC_{oral}) to that following intravenous administration (AUC_{iv}), as seen from Equation 4.2.1.

$$f_{oral} = \frac{AUC_{oral}/D_{oral}}{AUC_{iv}/D_{iv}} \tag{4.2.1}$$

D_{iv} and D_{oral} represent the intravenous and oral dose, respectively. However, oral bioavailability is also referred to as the fraction of the oral dose that reaches the systemic circulation. Thus, when drug-eliminating organs are arranged in series, such as the small intestine and liver (see Figure 4.2.2), bioavailability can be calculated as the product of the fractions of the dose that escape metabolism by each organ, where f_a is the fraction of an oral dose absorbed intact across the gut wall, and f_g and f_h are the fractions of the absorbed dose that escape metabolism by the intestine and liver, respectively. In fact, these three terms represent the most important properties that determine absorption after oral administration: permeability, solubility, and first-pass metabolism. The influence of these properties on the extent of absorption from the intestinal tract has attracted considerable attention (Amidon *et al.*, 1995; Norris *et al.*, 2000; Zhang

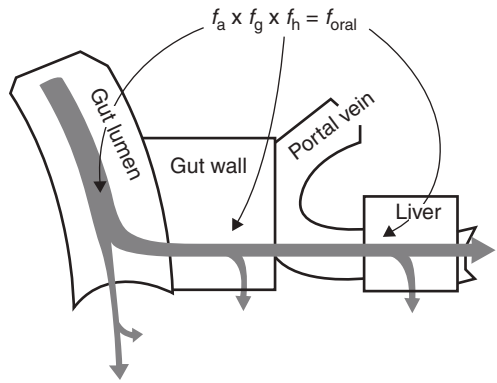

Figure 4.2.2 Schematic simplified view of the absorption process. Oral bioavailability (f_{oral}) is the product of the fraction of the drug dose absorbed into and through the gastrointestinal membranes (f_a), the fraction of the absorbed dose that passes through the gut into the hepatic portal blood unmetabolised (f_g), and the hepatic first-pass availability (f_h). The concept emerging is is that transporters would be involved with each of the absorption steps.

and Benet, 2001). Here, the discussion of these factors is divided into pre-membrane (before the drug passes across the mucosal membrane), and post-membrane (factors other than permeability and solubility). The interplay of all these factors will also be discussed.

4.2.1.2 Pre-membrane prediction

The introduction of combinatorial chemistry and high-throughput pharmacological screening in drug discovery has resulted in a vast number of lead compounds. The compounds generated from a high-throughput discovery programme are generally more lipophilic and of higher molecular weight than conventional drugs (Lipinski, 2000; Lipinski *et al.*, 2001). The rate at which a drug goes into solution is an important determinant for its absorption from the gastrointestinal tract. Factors affecting the kinetics of drug dissolution can be identified by the Noyes–Whitney equation (Equation 4.2.2):

$$\left(\frac{\mathrm{d}C}{\mathrm{d}t}\right)_{\text{drug}\rightarrow\text{solution}} = \frac{DS}{V}\left[\frac{C_S - C}{h}\right] \qquad (4.2.2)$$

where C is the concentration of solute in the bulk solution at time t; C_S is the solubility of the solid; t is time; D is the diffusion coefficient of the drug in solution; S is the surface area of the exposed drug particle and h is the thickness of the diffusion layer.

Dissolution depends on the surface area of the dissolving solid and the solubility of the drug at the surface of the dissolving solid. Dissolution is further described in Chapter 4.1 and solubility in Chapter 2.1. Considering surface area and solubility factors separately, surface area is manipulated by processing and formulation, whereas solubility is manipulated mainly by changing the chemical structure or salt of the candidate. The solubility of a drug candidate is inversely proportional to the number and type of lipophilic functionalities within the molecule. The dissolution rate of a drug candidate is affected by its solubility, i.e. its actual concentration in the aqueous phases of the gastrointestinal (GI) fluids. The concentration of drug candidate in solution determines the driving force through it, and thus its membrane transfer into the body. Formulation scientists primarily use dissolution to evaluate the properties of the drug itself and thereby select appropriate formulations. Clinical scientists primarily use dissolution tests to establish *in vitro–in vivo* correlations between drug release from the dosage form and drug absorption.

Simple approaches are used for identifying easily computed descriptors that may be applied in predicting intestinal absorption of leads and that can dramatically improve the speed of selection of lead compounds. One widely used approach is *Lipinski's 'rule of five'* (Lipinski *et al.*, 1997). It is proposed that membrane transfer may be limited by various factors; i.e. poor permeability is more likely when:

- there are more than five H-bond donors (sums of OHs and NHs)
- there are more than 10 H-bond acceptors (sums of Ns and Os)
- molecular weight is over 500
- there is poor dissolution resulting from log$P > 5$.

Lipinski's rule of five is described in more detail in Chapter 2.4. It should be kept in mind that substrates of any transporters are considered exceptions to the rule. Among several thousand drugs tested, some drug classes fell outside the rule of five: antibiotics, antifungals, vitamins and cardiac glycosides (Lipinski *et al.*, 1997). When the rule of five was developed, the knowledge about drug transporters was very limited. It is now believed that almost all drug substances are substrates for some transporter (Wu and Benet, 2005; Custodio *et al.*, 2008). Studies to date have not been able to show this because we are only just beginning to gain the knowledge and tools that allow investigation of substrates for absorptive and exsorptive transporters. In addition, unless a drug substance can passively gain intracellular access, it is impossible to simply investigate whether the molecule is a substrate for efflux transporters. Despite the fact that many

drug candidates are substrates for transporters, it is not uncommon for medicinal chemists in pharmaceutical industries to be advised to only optimise drug candidates by fine tuning the 'rule of five' factors.

4.2.1.2.1 The Biopharmaceutics Classification System

In 1995 Amidon *et al*. devised a Biopharmaceutics Classification System (BCS) to classify oral drug substances, based on their dose-relevant aqueous solubility and intestinal permeability, determined as extent of oral absorption (Amidon *et al*., 1995):

- class 1: high solubility, high permeability
- class 2: low solubility, high permeability
- class 3: high solubility, low permeability
- class 4: low solubility, low permeability.

The BCS was developed to serve as a regulatory tool for identifying those substances for which *in vivo*-based bioequivalence studies can be replaced by *in vitro* dissolution studies.

The recommended methods for determining solubility and permeability are discussed later in Section 4.2.2 (FDA, Center for Drug Evaluation and Research, 2000). Basically, the waiver of bioavalability/bioequivalence is only considered by the FDA for drugs with high solubility, high permeability and rapid dissolution (class 1). The replacement of *in vivo* bioequivalence studies by *in vitro* dissolution test studies yields certain benefits. The most rewarding benefit is minimisation of drug exposure in large numbers of volunteers. Other benefits are shortened development time and reductions of study costs. To summarise, the BCS addresses the following two factors of importance to oral drug bioavailability: (1) *drug solubility* identifies when limited solubility of a drug substance may give rise to incomplete release from the dosage form; (2) *drug permeability* addresses the efficiency of drug transport across the gut wall. However, a third important factor (3) is *drug stability or metabolism* such as chemical and enzymatic stability. Stability/metabolism should also be included in the BCS and this issue will be discussed in the next section.

4.2.1.2.2 Post-membrane prediction

Solute concentrations of a given drug substance within the GI fluids are generally much higher than its systemic concentrations, simply because its

volume of distribution within the GI fluids is generally much smaller than the total systemic volume. Therefore, an intravenously administered dose, as well as the absorbed fraction of an oral dose that reaches the systemic circulation, generally results in much lower drug concentrations at the eliminating organ compared to the concentrations in the intestine. Consequently, transporters and metabolising enzymes in eliminating organs and the drug solubility in eliminating organ fluids may be relatively unimportant, because saturation of transporters (and enzymes) will be minimal, if present at all, and solubility considerations will be unimportant because it is generally believed that only solutes are absorbed. The interactive roles of the enzymes and transporters in absorptive and distributive organs, such as the intestine and liver, become more important.

4.2.1.2.2.1 Intrinsic clearance

The most important factor, governing the extent and rate of first-pass metabolism, is commonly expressed by the Michaelis–Menten equation (Equation 4.2.3):

$$v = \frac{V_{max} \times S}{K_m + S} \tag{4.2.3}$$

where v is the rate of metabolism, S is the substrate concentration, and V_{max} is the maximal rate at which a drug substance is metabolised. K_m, the Michaelis constant, is the substrate concentration at which the rate of metabolism is one-half of V_{max}. The intrinsic clearance (CL_{int}) of a drug substance in an organ is defined as the maximum inherent efficiency of the organ in eliminating the substance (Wilkinson, 1987). CL_{int} may be determined by organ perfusion studies. The 'true' CL_{int} value will be obtained if there is no protein binding of the substance and/or if drug delivery to the organ is not limited by blood perfusion. CL_{int} is thus regarded as a measure for the organ 'eliminative activity'. Under linear conditions, CL_{int} can be obtained by measuring the rate of substrate disappearance (or rate of metabolite formation) at different substrate concentrations, i.e. when S is much smaller than K_m, CL_{int} is given by Equation 4.2.4:

$$CL_{int} \approx \frac{v}{S} \approx \frac{V_{max}}{K_m} \tag{4.2.4}$$

The concept of intrinsic clearance is important not only with regard to quantitative interpretation and prediction of drug interaction within the liver, but also with regard to prediction of pharmacokinetic parameters in general.

4.2.1.2.2.2 *In vivo* clearance

In vitro hepatic CL_{int} is most commonly used for *in vitro–in vivo* correlation (Houston and Carlile, 1994, 1997; Obach *et al*., 1997). The hepatic clearance after an intravenous dose is usually used for determining the *in vivo* hepatic clearance. However, in comparison between methods, i.e. from *in vitro* microsomes to whole organ (*in vivo*), interplay between the cell membrane and intracellular metabolic events should be taken into consideration. For example, hepatic uptake and efflux transporters play important roles in drug disposition and metabolism. When considering the transporter effect on hepatic CL_{int}, it may be more correctly determined by using methodologies that preserve the transporter/enzyme architecture of the liver (e.g. hepatocytes, sandwich-type hepatocytes, perfused liver etc.) rather than methods where the architecture is not preserved, such as in microsomes. Uptake and efflux transporters may modulate a drug candidate's metabolism by altering its accessibility to the metabolising enzymes, thus changing the estimated metabolic CL_{int}. The interplay of transporters and enzymes must be considered when defining the liver CL_{int} of a drug candidate (or its CL_{int} in other organs such as the intestine and kidney), as well as when evaluating potential drug–drug interactions. In general, *in vitro* microsomal studies that show metabolism changes for a drug candidate, when an interacting substrate is added, will be predictive for *in vivo* interaction between the compounds, but will not necessarily give a quantitative prediction. However, when an *in vitro* microsomal study shows no metabolic interaction, it cannot be concluded that an *in vivo* metabolic interaction will not occur, particularly for compounds where transporter–enzyme interplay can result in significant metabolism changes due to transporter inhibition.

4.2.1.2.2.3 Drug–drug interactions

Table 4.2.1 summarises the predicted changes in area under the blood concentration curve (AUC) for drug candidates that are substrates of uptake or efflux transporters.

Following oral administration of drug candidates, significant interactions will occur for candidates that are substrates for both intestinal enzymes (e.g. phase I and II enzymes) and intestinal apical efflux transporters (e.g. P-gp, MRP2, BCRP). This is because concomitant inhibition of the intestinal enzymes and the apical efflux transporter both lead to reduced gut metabolism in a way that can synergistically increase the AUC (see Table 4.2.1). It is, therefore not surprising that drugs removed from the market at the FDA's recommendation (i.e. terfenadine,

Table 4.2.1 Predicted direction of change in systemic AUC of drug/prodrug substrates when co-administered with inhibitors to enzymes and/or transporters expressed in the intestine

	Intestine	
	Absorptive transporter inhibited	*Eliminative transporter inhibited*
No enzyme inhibition	↓	↑
Metabolising enzymes inhibited	↔↓↑	↑↑
Drug-releasing enzymes inhibited (prodrugs)	↓↓	↔↓↑

mibefradil, cisapride, cerivastatin) are predominately orally dosed drugs that are substrates for both CYP3A and P-gp.

4.2.2 Application of the Biopharmaceutics Classification System

At its core, the BCS is based on experimental methods applied to permeability and solubility studies. The objective of the BCS is to predict the *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility. The definition of solubility and permeability is defined by FDA guidance (FDA, Center for Drug Evaluation and Research, 2000) as follows:

- *solubility*: a drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1–7.5. The volume estimate of 250 ml is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass (about 8 ounces or approximately 230 ml) of water
- *permeability*: the permeability is based indirectly on the extent of absorption (fraction of dose absorbed, not systemic bioavailability) of a drug substance in humans and directly on measurements of the rate of mass transfer across the human intestinal membrane. Alternatively, non-human systems capable of predicting the extent of drug absorption in humans can be used (e.g. *in vitro* epithelial cell culture methods). A drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose.

The BCS system is based on the understanding that dissolution from the dosage form depends considerably on the *solubility* of the drug substance, and that absorption from the GI tract is dependent on *permeability* properties of the drug substance. However, dissolution is also affected by the formulation. Absorption from the intestine may be influenced by GI transit time or membrane permeability. Consequently, a waiver of bioequivalence studies may only be granted for products where more than 85% of the ingredient is dissolved in 30 min in all physiological media.

4.2.2.1 Regulatory aspects

The BCS is primarily used to identify what drug candidates are appropriate for replacing *in vivo* study with *in vitro* dissolution testing, i.e. bioequivalence waiver. It is now accepted that if a BCS class 1 drug substance is released from the dosage form very rapidly *in vivo*, gastric emptying will become the rate-limiting process for drug absorption. Thus, bioavailability is not dependent on biopharmaceutical properties and therefore *in vivo* investigation may be waived. Additional criteria for biowaiver eligibility are that the drug needs to be stable in the GI fluids, and it should be a non-narrow therapeutic index drug. The general concept is that differences in bioavailability (rate and extent) may only be observed between two essentially similar (generic) products if the two dosage forms exhibit different *dissolution*. However, this statement is only valid as long as the release from the dosage form represents the rate-controlling process for drug absorption. On the other hand, if the permeation through the intestinal membrane is rate limiting, dissolution properties may be of negligible importance. BCS class 3 drugs are also proposed for waiver of bioavailability/bioequivalence studies (Blume and Schug, 1999) because they are also characterised by high solubility, and because the bioavailability is less dependent on the release properties of the formulation than on the *in vivo* permeability. The reasoning for suggesting class 3 drugs for waivers of bioavailability/bioequivalence studies was that if two drug products, containing the same drug substance, have the same concentration–time profile at the intestinal membrane surface, then they will have the same rate and extent of bioavailability. However, this might not be true because it is obvious that for drug substances of class 3, excipients from the drug formulation may affect uptake transporters and consequently modify bioavailability (Wu and Benet, 2005). Similar conclusions can be reached for class 1 drug substances: if their dissolution is rapid under all physiological pH conditions, it can be expected that they will

also behave like an oral solution *in vivo*. Consequently, generic products of BCS class 1 and 3 drugs with different *in vitro* dissolution will not necessarily exhibit different *in vivo* performances, and vice versa. Until more is known about the importance of intestinal transporters, and until validated methodologies to predict the effects of formulation excipents on these transporters have been developed, expansion of *in vivo* bioequivalence waivers beyond class 1 substances is unjustifiable.

4.2.2.2 Characterisation of candidate drugs

Permeability and solubility are of key importance in the selection of drug candidates for further development. Molecules with poor permeability and/or solubility usually have low and variable bioavailability, which becomes the hurdle to be dealt with before the molecule can reach the pipeline. Experimental methods and relevant acceptance criteria, regarding permeability and solubility, are needed during the early drug discovery phase. Such procedures have also been introduced into the industry, including high-throughput solubility and permeability screening. It has even been suggested that the drug substances listed in the BCS classes 1 and 2 are eliminated primarily via metabolism, while class 3 and 4 compounds are primarily eliminated unchanged into the urine and bile (Wu and Benet, 2005). The simple categorisation under the BCS has revealed the fact that the high permeability of the class 1 and 2 drug substances means they are also readily accessible to the metabolising enzymes within hepatocytes. Figure 4.2.3 shows the characteristic relationship between BCS classes and drug disposition. It is generally believed that chemicals are biotransformed to become more polar, thus becoming

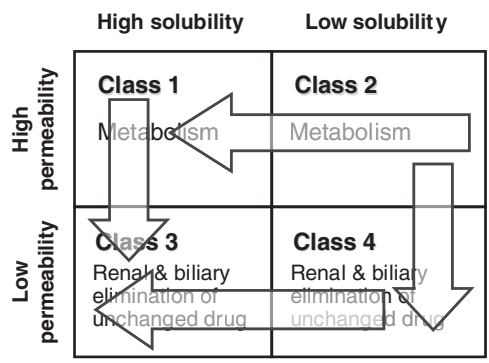

Figure 4.2.3 Predicted routes of drug elimination based on BCS categories. The arrows indicate the direction of biotransformation.

more feasible for excretion pathways. For compounds with certain molecular weights (human: >500, rat: >350), biliary secretion may become a major excretion pathway. Note that the different permeability classes defined in the BCS do not necessarily reflect corresponding differences in permeability into hepatocytes, but rather reflect differences in access to the metabolising enzymes within the hepatocytes. Therefore, the BCS classification may correlate with the disposition characteristics of the drug substances. Certain drug substances are primarily eliminated through a metabolic pathway, the rest are removed through excretion by either biliary or renal elimination

4.2.2.3 Formulation strategy

The BCS could be used as a framework to decide which types of formulation strategies are suitable for further development of a drug candidate. For candidates classified with low solubility, it is clear that bioavailability properties could be improved by formulation strategies that increase the dissolution rate and/or drug solubility, e.g. choosing the most water-soluble salt form, selecting the most soluble polymorph/anhydrate crystal form, providing the amorphous form with the most rapid dissolution, increasing solubility by super-saturation, or increasing the surface area of the crystals by micronisation, or by forming microemulsion, or nanoparticles. The optimal formulation should then provide a dissolution property that is no longer the rate-limiting step in the absorption process, i.e. situations that are comparable to those of class 1 and 3 compounds. The most straightforward way to achieve non-dissolution rate-limited absorption is to formulate the drug candidate in a solution. However, for different reasons, such as aqueous stability, it is not always feasible to formulate solutions, but rather solid dosage forms such as tablets and capsules are produced. Note that the influence of above-mentioned dissolution-enhancing strategies on biopharmaceutical classification are illustrated in Figure 4.2.4. It is very common to modify the absorption properties of low-solubility drug substances, by physical formulation strategies, into classes 1 or 3. However, it would be difficult to modify low-permeability properties by physical formulation approaches, from class 4 to class 2, or from class 3 to class 1. As illustrated in Figure 4.2.3, biotransformation alters the chemical properties of drug substances as well as their elimination characteristics (metabolism versus excretion). This is why chemical formulation approaches, such as a prodrug approach, can move candidate compounds vertically in Figure 4.2.4, i.e. between classes 3 and 1 or classes 4 and 2, as well as horizontally between

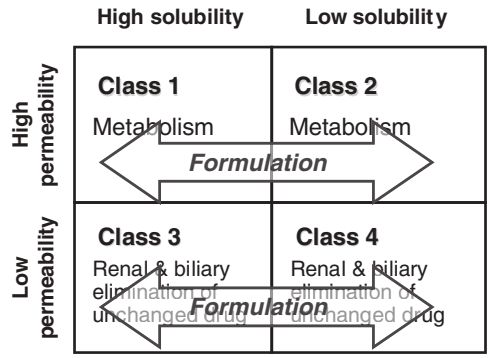

Figure 4.2.4 Formulation approach based on BCS categories. The arrows indicate the formulation strategy between different BCS class compounds.

classes 1 and 2 or classes 3 and 4, whereas physical formulation approaches can only move drug candidates horizontally in Figure 4.2.4, i.e. between classes 1 and 2 or classes 3 and 4.

4.2.2.4 Food–drug interactions

Alterations of bioavailability due to concomitant food intake can have serious implications for the clinical usefulness of a drug, and this is always in focus for clinical pharmacology profiling during drug development. It is well known that food can influence drug bioavailability, by changing the *extent* and the *rate* of availability. In December 2002, the FDA issued a guidance entitled *Food-effect Bioavailability and Fed Bioequivalence Studies* (FDA, Center for Drug Evaluation and Research, 2002). Fleisher *et al.* (1999) noted that food effects on the extent of bioavailability of drug substances could generally be predicted based on their BCS class. This observation that exposure of highly permeable and poorly soluble BCS class 2 compounds tends to be increased by high-fat meals, may be explained by increased GI fluid volume, which arises from co-administered meals stimulating GI secretions, and/or from biliary solubilisation that increases the dissolution rate. Alternatively it may be explained by food–drug interactions with transporters of BCS class 2 or 3 compounds (Wu and Benet, 2005). The fact that many water-soluble BCS class 3 substances show a sharp decrease in absorption when co-administered with a meal may either be explained by the food making a simple additional physical barrier that compromises simple diffusional permeability across the upper intestinal membrane, or by interference with the uptake mechanism between food and

class 3 drug substances. High-fat meal studies are recommended by the FDA, since such meal conditions are expected to provide the greatest effects on GI physiology, so that systemic drug availability is maximally affected and observed (FDA, Center for Drug Evaluation and Research, 2002).

4.2.2.5 *In vitro–in vivo* correlation (IVIVC)

In vitro dissolution testing is an important tool in the development of solid drug products, as well as in batch quality controls. The aim of the dissolution test is to see that the drug is appropriately dissolved in the GI tract and made available for absorption. It is therefore highly desirable that the *in vitro* tests provide data that correlate well to the *in vivo* situation. Drug dissolution and intestinal permeability are the fundamental parameters governing the rate and extent of drug absorption and the expectations regarding *in vitro* dissolution and *in vitro* absorption (IVIVC) are summarised in Table 4.2.2. It is important to realise that the *in vitro* dissolution test only models the release and dissolution of the drug substance from the formulation, and it is only when these processes are rate limiting in the absorption process that IVIVC can be expected. In the case of class 1 drugs, the complete dose will already be dissolved in the stomach and, provided that absorption across the gut wall is negligible, the gastric emptying of the dissolved drug will be rate limiting for absorption. Thus, no IVIVC should be expected for class 1 compounds as long as the release of drug is faster than gastric emptying. Class 3 compounds exhibit a high variability in the rate and extent of absorption, but if dissolution is fast (such as 85% of drug dissolving in 15 min), the variation could be attributed to GI transit, luminal contents, and membrane

Table 4.2.2 IVIVC expectations for immediate-release products based on the BCS

BCS class (rate-limiting step)	*IVIVC expectations*
1: gastric emptying	No IVIVC until product dissolution becomes rate-limiting step
2: dissolution	IVIVC expected provided that *in vitro* relevant dissolution test methods are used and drug absorption is limited by dissolution rate rather than by saturation solubility
3: permeability	No IVIVC until product dissolution becomes rate-limiting step
4	Limited or no IVIVC

permeation rather than dosage form factors. In this case, limited or no IVIVC is expected.

For class 2 compounds, a strong correlation between dissolution rate and the *in vivo* absorption could be difficult to establish. For example, if a rapid and complete dissolution of a class 2 compound is achieved by formulation approach, gastric emptying rather than dissolution becomes the rate-limiting step. Thus, it would be comparable to class 1 compounds. That is why no correlation would be expected in such a case. Another reason could be if absorption is limited by the saturation solubility in the GI tract rather than the dissolution rate. In this situation, the drug concentration in the GI tract will be close to the saturation solubility, and changes of the dissolution rate will not affect the plasma concentration–time profile, and consequently not the *in vivo* bioavailability. Standard *in vitro* dissolution tests are carried out under 'sink conditions', i.e. at concentrations well below the saturation solubility. Thus, only effects related to the dissolution rate can be predicted *in vitro*; the dissolution profile for class 2 compounds requires multiple sampling times and the use of more than one dissolution medium. For a compound that is primarily eliminated through a metabolic pathway, the other factors contributing to poor IVIVC of class 2 compounds are the interplay between transporters and enzymes. Therefore, a successful IVIVC can only be obtained after establishing a thorough understanding of the physicochemical properties as well as the uptake and elimination mechanism of the compounds.

4.2.3 The Biopharmaceutics Drug Disposition Classification System

The Biopharmaceutics Drug Disposition Classification System (BDDCS) was developed by Wu and Benet (2005) to predict the *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility. The major difference between the BCS and BDDCS is the interpretation of the permeability term, which is looked upon, and consequently determined as, the extent of oral absorption in the BCS, but as the extent of metabolism (access to metabolic enzymes) in the BDDCS. In the BDDCS, the extent of metabolism is divided into drug substances that are extensively metabolised, i.e. $\geq$70% metabolism of an oral dose *in vivo* in humans, and those that are poorly metabolised; i.e. $\geq$50% of an oral dose *in vivo* in humans is excreted unchanged (Custodio *et al.*, 2008).

The BDDCS may be useful for prediction of overall drug disposition, including routes of drug elimination; the effects of efflux and

absorptive transporters on oral drug absorption; when transporter–enzyme interplay will yield clinically significant effects (e.g. low bioavailability and drug–drug interactions); the influence, mechanism and importance of food effects; and transporter effects on post-absorption systemic drug concentrations following oral and intravenous dosing. These predictions are based on a series of studies, over the past few years, in which the effect of transporter inhibition and induction on drug metabolism was investigated (Wu and Benet, 2005).

The author considers the difference between the BCS and BDDCS to be substantial. Thus, the fundamental interpretations of permeability behind the two classification systems are very different, i.e. the extent of oral absorption versus the extent of metabolism. However, the outcome from the two systems seems to be similar since, as Takagi *et al.* (2006) have pointed out, the provisional classification of the top 200 oral products on the world market is similar in the BCS and BDDCS. Thus, when the drug substance cimetidine is used as a reference for permeability, the distribution of 164 drug substances in the BCS is, respectively, 61:58:38:7 for class 1: class 2: class 3: class 4. This is very close to the distribution in the BDDCS, which is, respectively, 59:51:42:12. However, the classification part of the BDDCS is only of minor value, whereas the implication part of the BDDCS is the most innovative and valuable, because it may be applied for much more than biowaiver justification. The application of the BDDCS is described in detail in the original commentary paper as well as by Custodio *et al.* (Wu and Benet, 2005, Custodio *et al.*, 2008).

4.2.3.1 The BCS from the point of view of drug disposition

As discussed earlier, Amidon *et al.* (1995) proposed using the BCS to categorise drugs into four classes according to their solubilities as well as their permeabilities through GI mucosa. Besides the regulatory applications of the BCS, it is also applied in the development process. The main merits of the BCS and BDDCS are the very clear and simple rules by which oral absorption and the extent of metabolism respectively are determined. In fact, the classification reflects the industrial optimisation process, i.e. the screening stage in new drug development. Until recently, mathematical modelling was widely used to explain and quantify the effect of a structure change on a defined biological activity.

In early drug development, the properties of candidates are always characterised to optimise pharmacokinetic performance. A typical example is depicted in Figure 4.2.5a, which shows that leads with optimal

pharmacokinetics usually have a medium partition coefficient. Properties of drug substances, such as permeability, solubility and metabolism that affect human absorption, can also be discussed in relation to the BCS. Lipinski and co-workers pointed out that identified leads from high-throughput screening (HTS-leads) tend to have higher molecular weight and lipophilicity than leads identified in the pre-HTS era (Lipinski *et al.*, 1997). In BCS terminology, this means that HTS leads tend to have limited dissolution and mainly belong to BCS class 2 (see Figure 4.2.5a).

Drug substances that are metabolised by, for example, CYP3A may have to be highly permeable in order to be metabolised within intracellular compartments. Consequently they may generally be classified as 1 or 2 in the BCS as illustrated by a darker shade in Figure 4.2.5b.

It has been suggested that drug substances should be passively *permeating* cell membranes, at least moderately, in order to be effluxed by P-gp. Such drug substances are, driven by concentration gradient, able to enter the cell by passive diffusion, but effective P-gp efflux can compete

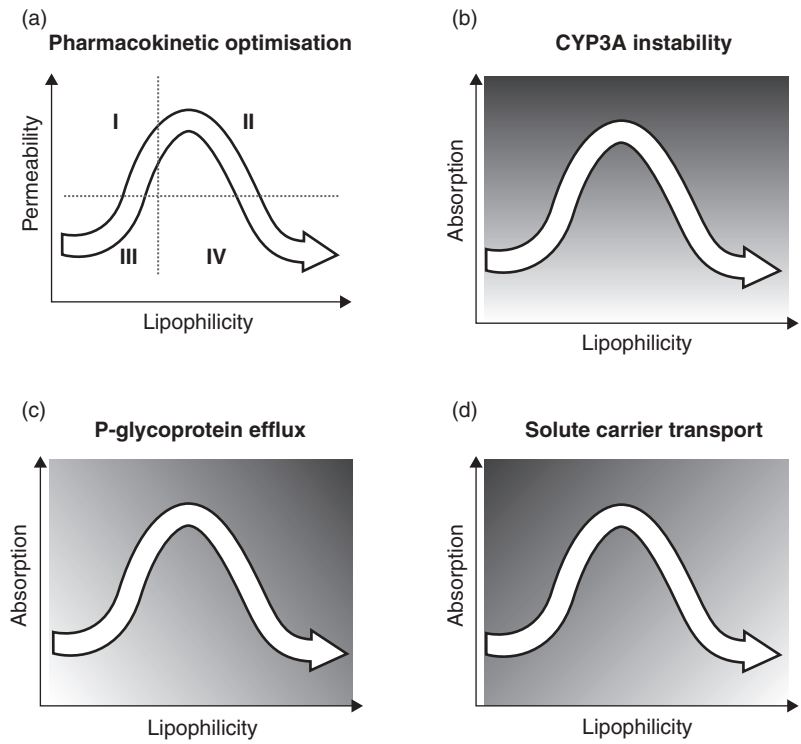

Figure 4.2.5 The relationship between the BCS/BDDCS and drug disposition in terms of metabolic stability, P-gp efflux and solute carrier transport potential.

against its passive permeability. BCS class 1 compounds such as midazolam, with rapid transmembrane movement, may pass through the cell more quickly than P-gp can remove it (Tolle-Sander *et al.*, 2003). In contrast, compounds such as ranitidine (BCS class 3), whose passive permeability is low, may never reach the intracellular concentrations needed to be effluxed by P-gp. This is in agreement with the fact that the BCS class 2 apparently contains many P-gp substrates (Wu and Benet, 2005). Thus, in order to be P-gp substrates, drug substances should permeate biomembranes, at least moderately, by passive diffusion (Lentz *et al.*, 2000; Faassen *et al.*, 2003) and these compounds are generally characterised as lipophilic, which corresponds to P-gp substrates tending to be located in BCS class 1 or 2 as illustrated in Figure 4.2.5c by dark shading. Solute carrier substrates, on the other hand, are more hydrophilic and water soluble and need specific transport mechanisms to be efficiently absorbed. Such highly water-soluble drug substances tend to belongs to BCS classes 1 and 3. This is illustrated in Figure 4.2.5d, by darker shading in classes 1 and 3. Figure 4.2.5 illustrates not only that the BDDCS is analogous to a pharmacokinetic optimisation process during pharmaceutical profiling, but also that lead identification by lead-HTS in drug development will result in more BCS class 2 compounds being developed and that this class of leads will have the highest risk of being substrates of CYP3A and P-gp. That partially explains why CYP3A substrates comprise half of the market's available drug substances, and also why there is a striking overlap in the substrates listed in both CYP3A and P-gp substrates (Wacher *et al.*, 1995).

The rationale for using use permeability and solubility as parameters in the BCS for biowaiver of a bioequivalence study is derived from the fact that these two parameters directly determine the oral absorption profile of drugs. Consequently, IVIVC of bioavailability/bioequivalence can be discussed and evaluated based on those parameters. In the BDDCS classification criteria, 'permeability' is determined by the *extent of metabolism*, in contrast to the BCS which uses the *extent of oral absorption*. The importance of the BDDCS is not limited to a regulatory application to investigate the possible relationship between *in vitro* drug permeability and *in vivo* bioavailability. The scientific fact is that the extent of metabolism can be viewed as a subsequent step to permeability, another surrogate marker for predicting absorption; this is related to the permeability of drug substances, and their consequent access to metabolising enzymes, and further to the intracellular events that take place thereafter. The future application of the BDDCS in drug development may become more widespread when the present framework gains

increased recognition. This will probably be the case if the interplay of transporters and enzymes become better recognised. The BDDCS is a simple concept and tool that can be used in early drug development not only to determine the rate-limiting step in the oral absorption process, but also to better predict drug disposition in possible drug substance interactions with transporters and enzymes in the ADME process.

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4.3

Biosimulation studies

Isabel Gonzalez-Alvarez and Marival Bermejo

Mathematical models and simulations are powerful tools in all phases of medicine development, from drug discovery to clinical phases. Nevertheless, the pharmaceutical industry is just starting to implement biosimulation for registration purposes. The aim of this chapter is to describe the basic concepts about modelling and simulation within the framework of preclinical investigations and *dosage form evaluation*. As an example, some models developed for oral drug administration will be described because this route is the most used. However the same modelling concepts can be applied to any other administration route. This chapter is not intended to be comprehensive, but to give an overall view of the potential use of modelling and biosimulation in dosage form evaluation. In some cases, the reader is referred to papers in which the original methods are described, while in others the reader may be directed to the latest publications in which the methods are applied.

4.3.1 What is modelling and simulation?

The first step is to establish simple definitions of modelling and biosimulation in the context of dosage form evaluation. Thus, in this context, *modelling* is constructing a mathematical description of a system such as an oral route of drug administration. The model can then be used to simulate or optimise the system. All models are predictive, i.e. the simulation output predicts what could occur in the real world where the system is operating (Nørager *et al.*, 2005). In other words, a mathematical model explains the behaviour of a system such as the oral of drug administration route by functions and equations that may describe the relationship between variables representing the properties of the system. System variables of the oral route of drug administration are further presented in Section 4.3.3. The variables representing these properties can be measured outputs, time data, event occurrence, etc.

There are a couple of comments to add to the above model definition. First, the model is defined not only by equations or mathematical functions but also by a series of underlying assumptions that must be meaningful to ensure the model validity. Examples are the distribution probabilities used to describe the variation of random errors; the function probabilities associated with the system variables; or the mechanistic or empirical assumptions used to construct the model. Second, the model can be used to make predictions that depend on its empirical or mechanistic nature. In general, an empirical model uses mathematical equations to describe and reproduce experimental observations. It can therefore be applied within the range defined by the experimental data that have been used to construct the model, and the model can be useful for interpolation, but it will be less reliable outside these limits. A mechanistic model, based on some previous knowledge about the process, could be used for extrapolation. However, it should be used with caution, especially if the hypotheses supporting the model are applied outside the experimental data range.

Simulation is imitation of reality. In the context of dosage form evaluation, simulation is using a mathematical model to predict what will be the outcome of a given input in reality. Simulation can thus be used to study the effect of changing parameters in a model or to predict experimental results, without actually performing a physical experiment. It can be seen as numerical evaluation of a model system and it may be used to estimate the true characteristics of the system. A simulation is, in other words, an experiment that is run as a model of reality (Norager *et al.*, 2005).

Models can be classified in several ways. Examples of classifying models by, for example, types of equations are shown in Table 4.3.1.

Modelling and simulation allow the scientist to condense experimental data into groups of parameters, and to predict the response of a system to changed parameters. That implies prediction of outcome from different experimental designs and, eventually, one may avoid execution of data from less well-designed experiments. Of course the confidence of the predictions depends on the accuracy of previous assumptions and on the accuracy of the model itself. In this way, modelling and simulation should be seen as feedback processes. Thus, as new experimental data become available, the knowledge generated from these data should be incorporated in the model to refine it in a continuous fashion.

In dosage form development, models can be used for different purposes. If the starting point is an already accepted model, then the parameter values can be estimated from experimental data. With these values, as

Table 4.3.1 Classification and examples of mathematical models (adapted from Endrenyi, 1981)

Classification	*Type of model*	*Example of equation and particular appplication*	
Type of equation	Linear	$y = a + bx$	Objective functions and constraints are linear
		Calibration curves	
	Non-linear	$y = m^*(1 - e^{-b^*x})$	Any of the objective functions or constraints are non-linear
		First-order dissolution profile	
	Integrated	$y = y_0{}^*e^{-k^*x}$	
		Plasma profile after iv dose of one compartment drug	
	Differential	$dy/dx = -k^*y$	
		Plasma profile after iv dose of one compartment drug	
Presence of error terms, noise or randomness	Deterministic	$y = a + bx$	Performance is always the same for a set of initial values, parameters etc
	Stochastic	$y = a(f(\phi)) + b(f(\phi))x + \varepsilon$	It incorporates some randomness either as a noise $f(\phi))$ in the parameters or as a random error term in the output
Time dependence	Static	$y = a + bx$	Describe a process/ system not time dependent
		$x \neq t$ and a and b are not $f(t)$	
	Dynamic	$y = y_0{}^*e^{-k^*x}$	Time is one of the variables of the model
		$x = t$	
Dependent variable	Explicit	$y = y_0 - k^*x$	The dependent variable can be separated in one side of the equation

(*continued*)

Table 4.3.1 (Continued)

Classification	*Type of model*	*Example of equation and particular appplication*	
	Implicit	$y_0 - y_p + K_m \ln\left(\frac{y_0}{y_p}\right) = V_m x$	The dependent variable and the one or more independent variables are not separated on opposite sides of an equation
		$x = t$; dependent variable y_p	

well as a measurement of the uncertainty of the parameters and the residual variability, it is possible to obtain a *prediction* about the modelled property under different initial conditions. Finally, once the critical parameters determining the behaviour of a system have been characterised, it is possible to *optimise* the model performance by changing them. Typically, this is done to increase the process yield, achieve new specifications, or reduce/increase the process duration.

4.3.2 How to construct and verify a model

In this section, *inverse problems* will be described. To solve an inverse problem, one has to find the parameter values of the model that best describe the observed data. In other words, the objective is to identify the parameters of the input function that yield a given response. In this way, the modelling exercise is used as a tool to explore mechanisms and to generate a feasible hypothesis about the behaviour of the system, by means of comparing the goodness of fit indices of different models. The modelling procedure should include three steps: an exploratory data analysis (preliminary graphical analysis and statistical tests), model development and model validation (to validate its predictability). Detailed definitions of validation methods and references can be reviewed in the FDA population pharmacokinetic guidance (FDA, Center for Drug Evaluation and Research, 1999).

In general, the approach to solving an inverse problem is to reduce the overall difference between the experimental data points and the estimated points obtained by changing the values of the parameters. *These distances are used to calculate the objective function, which, in this case, has to be minimised (least squares regression).*

After the exploratory data analysis step, the second stage in a modelling approach is to select the dependent variable (the response or system property we want to predict), the independent variable(s) and the mathematical function(s) linking them, and, finally, the definition of the objective function. Linear and non-linear regressions by least squares are based on the following assumptions, which should be validated before model fitting:

- the selected model is the right one; if the model is not the right one, the lack of fit will be reflected in the residual sum of squares
- the independent variable (x) has no error; in general, it is enough if the error in the independent variable is smaller than with the errors in the dependent variable (y)
- the errors (true residual variability) are independent, and follow a normal distribution with mean zero and the same variance; in some cases, if any of these assumptions are not true, it is possible to transform the dependent variable or to select some weighting scheme (see below).

The results should be interpreted with caution if some of the assumptions do not hold true. Fundamentals of linear regression (that can be applied to non-linear regression) can be reviewed elsewhere (Montgomery *et al.*, 2001; Motulsky and Christopoulos, 2004). Exploratory data analysis permits validation of the assumptions and allow the necessary actions to be taken if the assumptions are not met (FDA, Center for Drug Evaluation and Research, 1999). The mathematics of objective functions are further described in Appendix 4.3.1.

4.3.3 How can a biosimulation model be applied for preclinical investigations and dosage form development?

The concept of biosimulation is applicable for any biological process. In the process of drug absorption, several models have been developed to describe the main events taking place in the intestinal lumen (i.e. dissolution and transit) and the drug permeation through the intestinal epithelium. Also other pharmacokinetic processes (metabolism, distribution, elimination) have been modelled in addition to the pharmacological response. The current trend in biosimulation is the systems biology approach which incorporates integrative mechanistic models with the final aim of whole-body simulation by using *in silico*, *in vitro* and *in vivo*

input data (Dokoumetzidis *et al.*, 2007). In this section, some of the earliest models developed for the oral absorption route are briefly described to help the reader understand the evolution of this discipline and how the individual pieces are being integrated in more sophisticated models and software

4.3.3.1 Modelling drug absorption

The oral route for drug administration is the most convenient, and preferred by patients and industry, so, in general, the main objective in the process of drug development is to develop drug products that are absorbed after oral administration, i.e. drugs with good oral bioavailabilities. This is a multifactorial problem that depends on three levels of parameters/factors. The first level of parameters arises from the physicochemical characteristics of the drug substance, i.e. solubility, pK_a, lipophilicity, particle size, molecular surface area, crystalline form and stability. These parameters are further described in Part 2. The second level of parameters arises from the drug formulation, i.e. dosage form, disintegration and dissolution (mechanism and rate). The dissolution parameter is further described in Chapter 4.1. Finally, the third level of parameters is physiological variables, i.e. gastrointestinal pH, gastric emptying, intestinal motility and transit time, intestinal secretions, intestinal blood flow, and membrane permeability. These parameters are further described in Section 3; however, for pH in various biological media see Chapter 2.1, Table 2.1.2. The drug must be released from the dosage form and get into solution, which is the first essential step before its absorption. For this reason, *solubility* (and dissolution rate) and *permeability* across the intestinal membrane can be identified as key parameters for a new chemical entity in order for it to become a lead compound. These factors constitute the fundamental aspects of the BCS described in Chapter 4.2, which has evolved into a modern tool to speed up the drug development process (FDA, Center for Drug Evaluation and Research, 2000). Different mathematical model approaches, incorporating dissolution and permeability, have been developed to predict oral absorption and oral fraction absorbed for drug candidates. For instance, in some models the main goal is to explore the correlation between drug absorption (expressed as fraction absorbed or drug permeability) and drug physicochemical parameters. These models are then used to select the best drug candidate from a group of compounds, i.e for *in silico* screening. The second approach consists of modelling the absorption process itself. This is done by incorporating some physiological variables

such as intestinal surface and considering the mass balance of the drug candidate at the absorption site. In the more advanced models, time events, such as gastrointestinal (GI) transit time are included as variables to predict plasma profiles after oral administration.

4.3.3.1.1 Molecular descriptors-based models to predict permeability and oral fraction absorbed

One of the simplest models is based on the so-called 'absorption potential' that is a parameter used for predicting the oral fraction absorbed. The absorption potential is based on the pH-partition theory of Brodie and co-workers (Shore *et al.*, 1957) but considers not only the drug pK_a but also other factors such as solubility and dose. The proposed equation is shown in Equation 4.3.1:

$$\mathrm{AP} = \ln\left(\frac{P \cdot F_{\mathrm{ni}}}{\mathrm{Do}}\right) \tag{4.3.1}$$

where AP is the absorption potential, P is the partition coefficient, F_{ni} the non-ionised fraction at pH 6.5 and Do the dose number (Do = Dose/ $250 * C_s$).

In order to establish a model describing the quantitative relationship between absorption potential and oral fraction absorbed (F_a), Macheras and Symillides (1989) proposed the following equation:

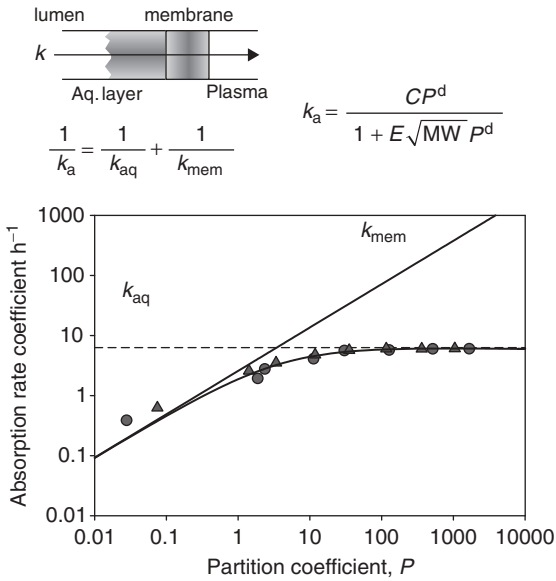
$$F_a = \frac{(10^{\mathrm{AP}})^2}{(10^{\mathrm{AP}})^2 + F_{\mathrm{ni}}(1 - F_{\mathrm{ni}})} \tag{4.3.2}$$

Assuming that AP has an upper limit of 1000 and if Do > 1, a value Do $= 1$ is used.

A second group of models derived from the pH-partition theory are the compartmental models from Wagner and Sedman (1973) and Higuchi and Ho (Higuchi *et al.*, 1981). In these models the lipid membrane and the aqueous media at both sides are both considered as compartments, and the diffusion from one compartment to the other is described as a function of the physicochemical characteristics of the compounds (mainly lipophilicity) (Wagner and Sedman, 1973; Plá-Delfina *et al.*, 1980; Higuchi *et al.*, 1981; Plá-Delfina and Moreno, 1981; Martin-Villodre *et al.*, 1986; Casabo *et al.*, 1987).

An example of the Higuchi–Ho model applied to a series of fluoroquinolones is shown in Figure 4.3.1.

In this model, the drug candidate absorption rate constant (k_a) depends on its diffusion rate constants through the aqueous stagnant



k_{aq} (h^{-1})	k_{mem} (h^{-1})
7.073	0.195
6.944	0.397
6.923	3.322
6.801	4.070
6.782	4.795
6.667	6.210
6.649	14.813
6.541	15.359
6.524	30.267
6.422	33.761
6.405	79.701
6.309	85.387
6.293	181.075
6.201	233.991
6.186	389.164
6.099	545.016

Figure 4.3.1 Absorption–lipophilicity correlation obtained for a group of fluoroquinolone compounds. The curve in the plot represents the fitted values to the Higuchi–Ho absorption model. The lines are obtained by decomposing the absorption process in the two diffusional steps through the aqueous layer k_{aq} and through the membrane k_{mem}. The values of k_{aq} and k_{mem} are indicated from the most hydrophilic to the most liphophilic compounds to point out how the diffusion through the aqueous layer becomes the limiting step as the lipophilicity is increased. The equations are described in detail in the text. Adapted from Merino *et al.*, 1995; Bermejo *et al.*, 1999).

layer (k_{aq}) and lipid membrane (k_{mem}). k_a is the sum of k_{aq} and k_{mem} as can be seen from the left-hand equation given in Figure 4.3.1. Diffusion through the aqueous boundary layer decreases from the most hydrophilic to the most lipophilic compounds in the series. The aqueous diffusional rate constant (k_{aq}) is expressed as a function of the molecular weight (MW); thus it is inversely proportional to the MW of the compounds as seen from Equation 4.3.3:

$$k_{aq} = J/\sqrt{MW} \tag{4.3.3}$$

where J is the inverse proportionality constant.

On the other hand, compound diffusion through a membrane is also quantified by means of its lipophilicity (P) as seen from Equation 4.3.4.

$$k_{mem} = CP^d \tag{4.3.4}$$

where C and d are the parameters linking the coefficient to the membrane permeability. This equation is based on the Collander relationship for the partition coefficients obtained using different organic solvents. Thus C and d are needed to link the partition coefficient in the *in vitro* system and the *in vivo* partition coefficient; the latter is generally not known or measured (Collander, 1951). Combining both equations gives Equation 4.3.5:

$$k_a = \frac{CP^d}{1 + E\sqrt{MW}\,P^d} \tag{4.3.5}$$

in which $E = C/J$

For more lipophilic compounds, diffusion through the stagnant water layer becomes the rate-limiting step, which leads to a 'plateau' in the absorption–lipophilicity correlation profile.

The model of Plá-Delfina and Moreno incorporates many of the principles described in the previous compartmental models; however, it also offers a global interpretation of the absorption–partition correlations which are obtained for many series of compounds when investigated in different intestinal segments (Delfina *et al.*, 1975, 1980; Plá-Delfina and Moreno, 1981). The main hypothesis, which is illustrated in Figure 4.3.2, is based on the assumption that intestinal absorption by passive diffusion is the result of two simultaneous processes described by the diffusion through aqueous channels (k_{a2}) (porous or tight junctions) and diffusion through the lipophilic membrane (k_{a1}). The correlation between absorption and lipophilicity can thus be represented by two hyperbolic functions, a direct one for the lipidic permeation and an inverse one for paracellular diffusion, with two asymptotic values, i.e. k_m for membrane permeation and k_p for aqueous diffusion. P represents the lipophilicity and the other symbols are parameters of the fit. This model can be applied to simulate the permeability of drug candidates across the small intestine, where both pathways are involved, and for candidates with MW low enough to use the aqueous paracellular permeability pathway. However, in the case of compounds with MW higher that 250 Daltons, and in the colon, the correlations are always hyperbolic. k_m represents the limiting value for the absorption rate constants of lipophilic compounds. This asymptotic value appears because the aqueous stagnant layer is the rate-limiting step in the diffusional process of more lipophilic compounds. Examples of these correlations are depicted in Figure 4.3.3 (Yu *et al.*, 1996).

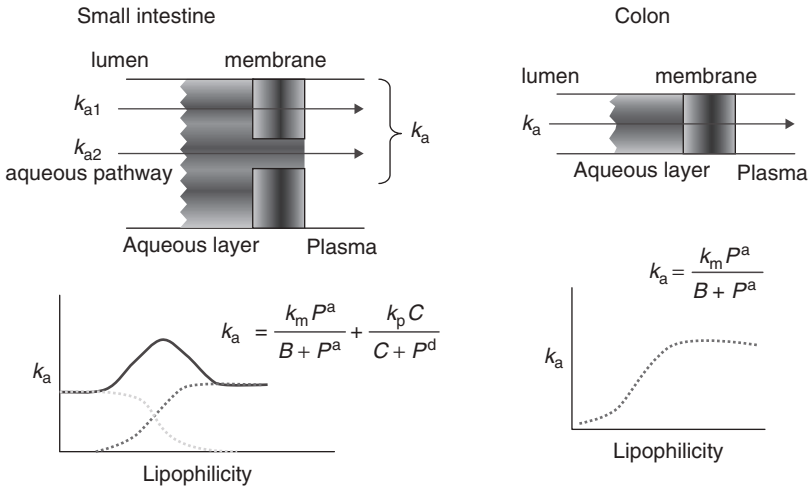


Figure 4.3.2 Scheme representing the main assumptions of the biophysical absorption model of Plá-Delfina and Moreno. Absorption versus lipophilicity correlations in the small intestine are described as the sum of two hyperbolic equations: a direct hyperbola for the membrane permeation, and an inverse hyperbola for paracellular diffusion. See text for explanation.

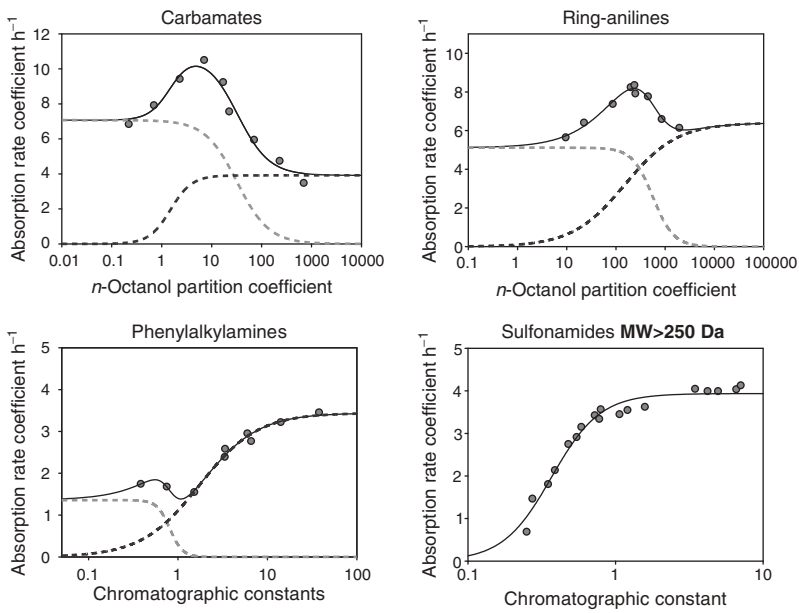


Figure 4.3.3 Examples of absorption–partition relationships obtained in rat small intestine for different families of compounds. Adapted from Plá-Delfina *et al.*, 1980; Plá-Delfina and Moreno, 1981; Martin-Villodre *et al.*, 1986; Casabo *et al.*, 1987.

Besides lipophilicity, other molecular descriptors such as MW, polar surface area, and hydrogen-bonding capacity have been used in the literature to predict ADME properties. Table 4.3.2 summarises some of these molecular descriptors and their relationship to oral absorption.

Table 4.3.2 Molecular descriptors used for drug-permeability predictions

Molecular descriptor	*References*
Lipophilicity: as drug partitioning into the cell membrane is one of the steps in membrane transport, lipophilicity is widely used as a predictor of drug permeability. Lipophilicity has two principal components, molecular size and hydrogen-bonding potential. This parameter has been used to predict *in vitro* permeability, and rat and human permeability using linear or hyperbolic correlations.	Casabo *et al*., 1987; Dowty and Dietsch, 1997; Kamm *et al*., 1999; Stenberg *et al*., 1999; Bermejo and Ruiz-García, 2002
Molecular weight: this is a component of lipophilicity as well as the diffusion coefficient in biological membranes and fluids. It has been included as predictor variable of oral absorption in multiple linear and non-linear models along with other descriptors. A rather strong dependence between transcellular diffusion and molecular size has been observed. Compounds with MW < 200 are able to pass through the intestinal membrane by paracellular pathways along with diffusion through the transcellular route. Compounds with MW > 250 use the transcellular route but further increases of the MW (MW>500) consequently lead to a decrease in membrane diffusion.	Camenisch *et al*., 1996
Hydrogen bonding capacity: the absorption ability of a molecule depends on the number and strength of the hydrogen bonds that the molecule is able to form with water molecules, because the first step to entering into the membrane is desolvation of the molecule. Hydrogen-bonding capacity is detrimental for transport into the non-polar environment of the cell membrane. Thus, this property as well as lipophilicity is a good descriptor of drug permeation and it has been included in multiple linear regression models to predict Caco-2 permeability and human oral fraction absorbed.	Norinder *et al*., 1999

(*continued*)

Table 4.3.2 (Continued)

Molecular descriptor	*References*
Polar surface area (PSA): PSA of a molecule is defined as the area of its van der Waals surface that arises from oxygen or nitrogen atoms plus the area of the hydrogen atoms attached to these hetero-atoms. As such, is clearly related to the capacity to form hydrogen bonds. Drugs with $PSA_d < 60\ Å^2$ would be completely absorbed (fraction absorbed, FA > 90%). Drugs with $PSA^d > 140\ Å^2$ would be absorbed less than 10%.	Clark, 1999a, b; Palm *et al*., 1996, 1997
Non-polar surface area: non-polar substituents facilitate membrane transport and hydrophobic compounds generally have higher permeabilities than hydrophilic ones (with similar hydrogen-bonding properties). Non-polar surface area can also correlate with membrane permeability. In general, this parameter is included in the correlations along with PSA.	

Yoshida and Topliss (2000) have studied the quantitative structure–bioavailability relationships of 232 structurally diverse drugs. They have identified a group of structural variables for their model and quantified their influence on absorption and/or metabolism, and have included lipophilicity (expressed as the distribution coefficient at pH 6.5) as a significant factor influencing bioavailability. A quantitative structure–activity relationship (QSAR) study, performed by Sakaeda *et al*. (2001) included over 222 commercially available drugs, and it showed exclusion criteria to differentiate poorly absorbed drugs which are similar to the exclusion criteria described by the rule of five presented by Lipinski (Lipinski *et al*., 2001). Essentially Lipinski's rule stated that a compound has a low absorption or permeability if it shows MW higher than 500 Da, a log*P* higher than 5, and there are >5 hydrogen bond donors (OH and NH groups) in the molecular structure and >10 hydrogen bond acceptors (notably N and O). Lipinski's rule of five is further described in Chapter 2.4.1.

4.3.3.1.2 Mass balance, time-independent models

In the simplest model it is assumed that the small intestine is a tube with area $S = 2\pi RL$, where R is the radius and L the length of the segment.

The mass balance in the segment can be described by Equation 4.3.6:

$$-\frac{dM}{dt} = \Phi\,(C_0 - C_f) = 2\pi\,R\,P_{eff}\int_0^L C\,dz \qquad (4.3.6)$$

in which M is the amount of drug absorbed, ϕ is the volumetric flow, C_0 and C_f are the concentrations at the beginning and end of the segment respectively, L is the length of the segment, R is the radius, P_{eff} is the drug permeability and z the axial distance.

At steady state, the fraction absorbed (f_a) is given by Equations 4.3.7–4.3.9:

$$f_a = 1 - \frac{C_f}{C_0} \qquad (4.3.7)$$

$$f_a = 2\frac{\pi\,R\,L\,P_{eff}}{\phi}\int_0^1 C^*\,dz^* \qquad (4.3.8)$$

$$f_a = 2\,A_n\int_0^1 C^*\,dz^* \qquad (4.3.9)$$

where C^* and dz^* are dimensionless variables that corresponds to C_f/C_0 and z/L. A_n is the absorption number, which is defined as the ratio between the transit time in the segment and the absorption time (R/P_{eff}). Table 4.3.3 summarises the solutions for Equation 4.3.9.

Table 4.3.3 Solutions to Equation 4.3.9

Comment	Conditions	Integral
Highly soluble drugs; permeability is the main parameter determining f_a	$C_0 \le C_s$ and $C_f \le C_s$	$f_a = 1 - e^{-2\,An}$
Drug in solid form (suspension). If dissolution is faster than absorption then concentration is C_s	$C_0 > C_s$ and $C_f > C_s$	$f_a = \frac{2\,An}{Do}$
	$C_0 > C_s$ and $C_f \le C_s$	$f_a = 1 - \frac{1}{Do}e^{-2\,An+Do-1}$

Notes: C_s is drug solubility, C_0 and C_f are the concentrations at the beginning and at the end of the intestinal segment respectively. An: absorption number; Do: dose number. See text for explanation.

This mass balance approach generally renders good predictions for high-solubility drug candidates, but for low-solubility candidates there are other more accurate approaches based on microscopic mass balance that provide better estimates (Oh *et al*., 1993).

4.3.3.1.3 Time-dependent models

The GI tract can be considered as one well-stirred mixing tank from which the drug is absorbed, or it can be represented by a series of tanks linked by linear transference processes. Several authors have constructed models following this scheme (Dressman *et al*., 1984; Dressman and Fleisher, 1986; Oberle and Amidon, 1987). An example of applying this approach is described below and represented in Figure 4.3.4.

Solid drug is assumed to have an initial distribution of particle size. Each particle size group is considered separately. Particles are considered to be spherical (but other geometries can be accommodated into the model). Particle dissolution is described by the Noyes–Whitney equation which is described further in Chapter 4.1. The diffusion layer thickness is assumed to be constant for particles with a diameter larger than 30 μm. The thickness is assumed to be equal to the particle radius for smaller

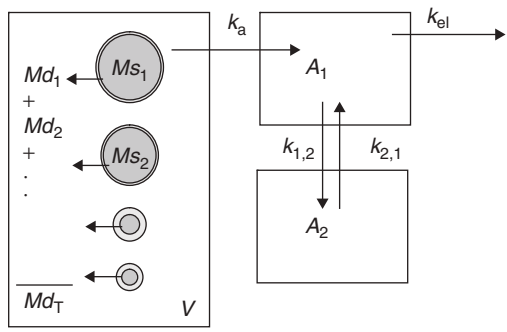


Figure 4.3.4 Scheme of a dissolution/absorption/pharmacokinetic model adapted from Johnson, 2003. Drug in polydisperse particles is either in solid form Ms_i or dissolved in the intestinal fluids Md_i, where i represents a fraction of particles (1, 2 . . . . n); V is the volume available in the intestinal lumen; A_1 and A_2 correspond to the drug mass in the central and peripheral pharmacokinetic compartments in the body; k_a is the first-order absorption rate constant and k_{el} the first-order elimination constant; $k_{1,2}$ and $k_{2,1}$ are the first-order distribution constants to and from the peripheral compartment respectively. Large particles have a diffusion layer of constant thickness, whereas particles smaller than 30 μm have a diffusion layer equal to the particle radius.

particles. The derivation of the dissolution equations is described in several papers (Dressman and Fleisher, 1986; Hintz and Johnson, 1989).

The following series of coupled differential equations is used to simulate the process of drug dissolution, absorption and disposition:

$$\frac{\mathrm{d}Ms_\mathrm{i}}{\mathrm{d}t} = -\frac{3D(M_{0\mathrm{i}})^{1/3}(Ms_\mathrm{i})^{2/3}}{\rho\, h_\mathrm{i}\, r_{0\mathrm{i}}}\left(C_\mathrm{s}(t) - \frac{Md_\mathrm{T}}{V(t)}\right) \tag{4.3.10}$$

$$\frac{\mathrm{d}Md_\mathrm{i}}{\mathrm{d}t} = +\frac{3D(M_{0\mathrm{i}})^{1/3}(Ms_\mathrm{i})^{2/3}}{\rho\, h_\mathrm{i}\, r_{0\mathrm{i}}}\left(C_\mathrm{s}(t) - \frac{Md_\mathrm{T}}{V(t)}\right) - k_\mathrm{a}(t)\, Md_\mathrm{i} \tag{4.3.11}$$

$$Md_\mathrm{T} = \sum_{\mathrm{i}=1}^{n} Md_\mathrm{i} \tag{4.3.12}$$

$$\frac{\mathrm{d}A_1}{\mathrm{d}t} = k_\mathrm{a}(t)\, F\, Md_\mathrm{T} - (k_\mathrm{el} + k_{1,2})\, A_1 + k_{2,1}\, A_2 \tag{4.3.13}$$

$$\frac{\mathrm{d}A_2}{\mathrm{d}t} = k_{1,2}A_1 - k_{2,1}A_2 \tag{4.3.14}$$

where Ms_i is the mass of drug in solid state in fraction I; $M_{0\mathrm{i}}$ the initial mass in the fraction; Md_i the mass of dissolved drug; D the diffusion coefficient; ρ the drug density; $r_{0\mathrm{i}}$ the initial radius of fraction I; h_i the thickness of the stagnant boundary layer around the particle; Md_T the summation of dissolved drug mass at any time from all particle size groups; $V(t)$ the dissolution volume; A_1 the mass of drug in the central compartment and A_2 the amount of drug in peripheral compartment; $k_{1,2}$ and $k_{2,1}$ are the distribution rate constants and k_el the elimination rate constant from the central compartment. $k_\mathrm{a}(t)$ represents the absorption rate constant, the parenthesis (t) means that the parameter can be made time dependent. This model was used to simulate the plasma profile of nifepidine in a gastrointestinal therapeutic system (GITS)-type dosage form, using the disposition parameters from iv administration (Johnson, 2003).

These mixing tank models are simple and intuitive but the main disadvantage is the lack of physical basis for assuming that one physiological segment of the small intestine can be considered as one homogeneous and well-stirred tank, although such an assumption has been commonly used in classical pharmacokinetic modelling. On the other hand, the number of mixing tanks will affect the results. To approach this issue, a mathematical model was developed in order to describe the transit flow of a drug in the human small intestine. Seven mixing tanks was found to be the best number for describing this process. The model

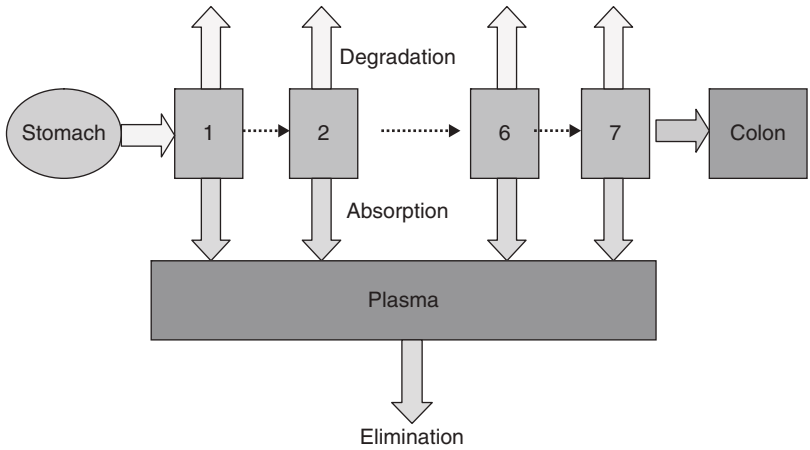


Figure 4.3.5 Schematic representation of the compartmental transit and absorption model (CAT). See text for explanation.

was named compartmental transit and absorption (CAT). The initial model incorporated linear intestinal absorption and degradation in the lumen (Yu *et al.*, 1996; Yu and Amidon, 1999). In another paper, the model was extended to account for saturable absorption (Yu and Amidon, 1998). The CAT model, along with intravenous pharmacokinetic parameters, was used to estimate not only plasma concentration–time profiles, but also the fraction of dose absorbed. The model is briefly described here and illustrated in Figure 4.3.5.

In the model, the small intestine is divided into seven segments, plus the stomach and colon. Each segment is considered as a compartment where the drug transits from one to the other (following a linear kinetic) and is absorbed. In the first CAT models, absorption from the stomach and colon was considered to be negligible and the drug dissolution was instantaneous (Yu *et al.*, 1996; Yu and Amidon, 1998, 1999). The differential equations describing the change of mass with time in each compartment are as follows:
Stomach:

$$\frac{dM_s}{dt} = -K_{se}M_s \tag{4.3.15}$$

K_{se} is the emptying rate constant and M_s a percentage of the dose.
Small intestine:

$$\frac{dM_n}{dt} = K_t\, M_{n-1} - K_a\, M_n - K_d M_n \tag{4.3.16}$$

M_n ($n = 1, 2 \ldots 7$) are the percentages of dose in each segment and K_t, K_a, K_d are the transit, absorption and degradation constants. In this case the constants are considered to be the same along the entire GI tract.

The rate of absorption can be defined from the absorption in each compartment:

$$\frac{dM_a}{dt} = \sum_{n=1}^{n=7} K_a M_n \tag{4.3.17}$$

In a similar way, the total amount degraded, M_d, can be estimated. The drug exiting from the last intestinal segment, M_c, passes to the colon, where absorption is considered to be negligible.

At time $t \to \infty$, the amount of drug in the stomach and intestine becomes zero, so:

$$100\% = M_a + M_c + M_d \tag{4.3.18}$$

The fraction absorbed is calculated by using the following expression:

$$f_{a\, t\to\infty} = \frac{M_a}{100} = \frac{\int_0^\infty \sum_{n=1}^{n=7} M_a K_a dt}{100} \tag{4.3.19}$$

The next step for predicting plasma profiles is to link the absorption process to a disposition compartmental model. For instance for a one-compartment drug:

$$\frac{dC}{dt} = \frac{D}{100}\left(\frac{dM_a}{dt}\right)\frac{1}{V} - k_{el}C \tag{4.3.20}$$

where k_{el} is the first-order elimination rate constant, V the distribution volume, C the plasma concentration and D the dose.

The original CAT model does not account for dissolution of the drug and the pH-dependent solubility of weak electrolytes. On the other hand, the absorption rate constant was modelled as a single parameter, without accounting for changes in factors such as surface area, transporter densities, efflux protein densities, and other regional factors within the intestinal tract. The CAT model can be made more accurate by treating the colon as an additional absorbing compartment. This applies in particular for low-solubility and low-permeability drugs and controlled-release formulations for which absorption in the colon can be significant. An example of application of the model to test the relevance of P-gp secretion in drug absorption has also been published (Yu and Amidon, 1999).

Modifications of the CAT model are implemented in simulation programs such as GastroPlus™ (based on the advanced CAT model (ACAT) (Agoram *et al.*, 2001)) and Simcyp© versions 7 and 8 (Advanced Dissolution, Absorption and Metabolism (ADAM) model).

The ACAT model includes allowance for changes in the transit constants from the upper to the lower segments. The model uses the concentration gradient across the apical and basolateral membranes to calculate the rate of drug transfer into and out of an enterocyte compartment for each lumen compartment, incorporating not only saturable absorption or efflux and intestinal metabolism but also the metabolic first-pass effect estimation. To estimate permeability values and the parameters of the metabolic processes, data coming from *in vitro* experiments can be used and they are scaled to the *in vivo* scenario using appropriate physiological scale factors.

The ADAM model, as implemented in Simcyp© Version 7, predicts the rates and extent of intestinal drug absorption and metabolism and their associated inter-individual variability (Dokoumetzidis *et al.*, 2007). The model is a population physiologically based mechanistic representation that accounts for the heterogeneity of the GI tract and considers the processes of dissolution, region-specific GI fluid dynamics, gut wall permeability and gut wall degradation and metabolism, with implicit consideration of active transport. The model incorporates a physiologically based treatment of fluid dynamics and basal fluid volumes within the GI tract, which includes the secretion and absorption of water for each ADAM intestinal segment. Physiological variability is applied to the GI tract surface area, transit times and fluid secretion/content/absorption in each segment by using a Monte Carlo approach. For dissolution, Simcyp© uses the Wang and Flanagan model (Wang and Flanangan, 1999) instead of the Noyes–Whitney model (Noyes and Whitney, 1897). Examples of application of the software to absorption of metoprolol and evaluation of the impact of P-gp have already been presented (Neuhoff *et al.*, 2008; Polak *et al.*, 2008).

An example with Gastroplus™ is shown in Figure 4.3.6. Data treated by the ACAT model are represented together with some plasma profiles predicted from physicochemical parameters and *in vitro* experiments, showing the good agreement of the simulated profile with the experimental plasma concentrations. On the other hand, the application of these modelling packages to the available clinical and preclinical data is extremely useful for model validation and refinement (Allan *et al.*, 2008).

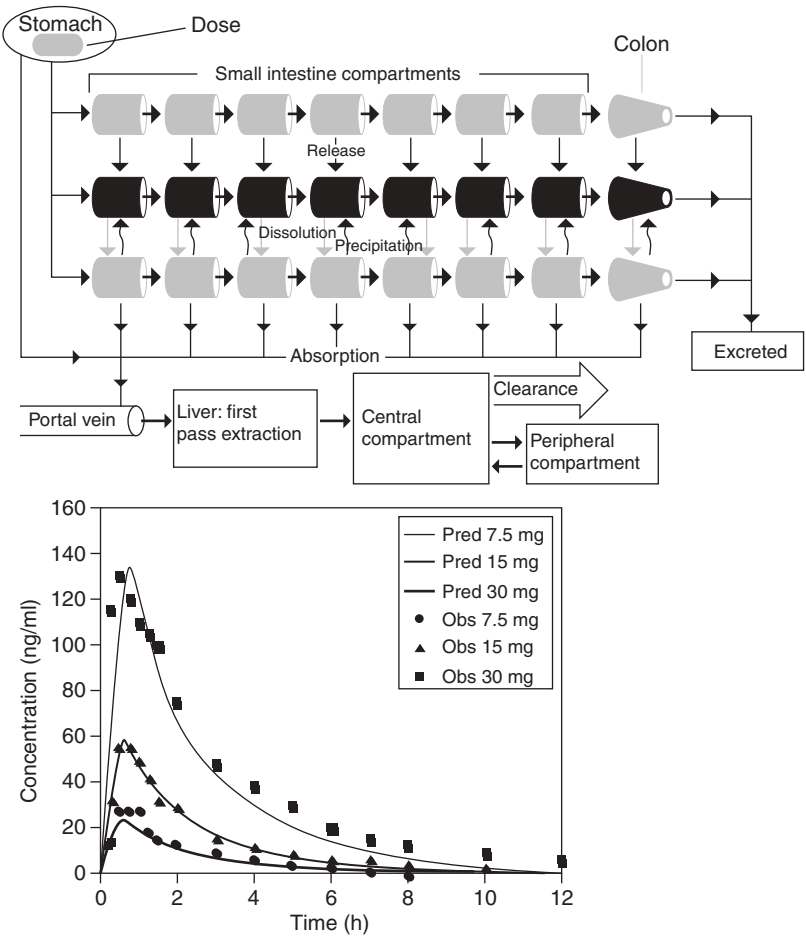


Figure 4.3.6 (a) ACAT model schematic. The original CAT model with seven compartments was modified to include compartment-dependent physiological parameters and the colon. One to three compartment pharmacokinetic models were also included to estimate C_p–time profiles. (b) Predicted plasma profiles simulated for midazolam (experimental data from Bornemann *et al.*, 1985) as the dose is increased by a factor of 4, bioavailability increased from 25% to 38%. Reprinted from Agoram B, Woltosz WS, Bolger MB (2001). Predicting the impact of physiological and biochemical processes on oral drug bioavailability. *Adv Drug Deliv Rev* 50 (suppl 1): S41–S67, Copyright (2001), with permission from Elsevier.

4.3.3.2 Modelling drug release and dissolution

There are numerous examples in the literature showing the critical role of drug dissolution on the rate and extent of absorption. The use of a mathematical model that relates some parameters of the dosage form to

the release of the drug and its dissolution facilitates the interpretation of the results of *in vitro* dissolution tests.

In some cases, the equations come from theoretical analysis of the process, as, for example, in zero-order or cubic root law kinetics. In many cases, other empirical equations are used. Table 4.3.4 summarises the equations (Equations 4.3.21–4.3.32) used to describe the dissolved amount of drug as a function of time. The theoretical bases for some of these equations are reviewed in detail by Costa and Sousa Lobo (2001).

Recently Dokoumetzidis and co-workers developed modified versions of the Noyes–Whitney and Weibull equations, explicitly including the solubility/dose parameter, for the analysis of dissolution data (Dokoumetzidis *et al.*, 2006). They applied these equations to metoprolol and ibuprofen dissolution and showed that the new equations performed better than the classical versions. On the other hand, the modified Weibull equation presented has a mechanistic meaning, as opposed to the purely empirical character of the original one.

According to the Noyes–Whitney equation, the rate of dissolution of a solid depends (among other factors) on its solubility and diffusivity in the dissolution media and on the surface area of the solid (see also Chapter 4.1). For drugs of low aqueous solubility, particle size (which determines the surface area) can have a significant impact on the dissolution rate. The drug in the dosage form is not generally present as a monodisperse powder but rather as a polydisperse system with a particular particle size distribution, which in most cases is log-normal. Hintz and Johnson (1989) developed a model for handling polydisperse particle dissolution based on a previous model by Dressman and Fleisher (Dressman *et al.*, 1984; Dressman and Fleisher, 1986,). This model generated Equation 4.3.33:

$$-\frac{\mathrm{d}Q^{\mathrm{i}}_{\mathrm{solid}}}{\mathrm{d}t} = \frac{3(Q^{\mathrm{i}}_0)^{1/3}(Q^{\mathrm{i}}_{\mathrm{solid}})^{2/3}}{\rho\, h\, r^{\mathrm{i}}_0}\left(C_{\mathrm{s}} - \frac{Q^{\mathrm{T}}_{\mathrm{diss}}}{V}\right) \tag{4.3.33}$$

in which $Q^{\mathrm{i}}_{\mathrm{solid}}$ is described by Equation 4.3.34:

$$Q^{\mathrm{T}}_{\mathrm{diss}} = \sum_{i=1}^{n} Q^{\mathrm{i}}_{\mathrm{solid}} \tag{4.3.34}$$

where $Q^{\mathrm{i}}_{\mathrm{solid}}$ represents the mass of solid drug in size fraction i, Q^{i}_0 the initial amount of drug in that size fraction, C_{s} is the drug solubility, V the volume of dissolution media, ρ the drug density, r^{i}_0 the initial average radius of particles in fraction i, h the thickness of the diffusion layer (controlling the dissolution rate), and finally $Q^{\mathrm{T}}_{\mathrm{diss}}$ is the total amount

Table 4.3.4 Dissolution models

Model	*Equation*	*Parameters*
Zero order	$Q_t = Q_0 + K_0 t$	K_0
First order	$Q_t = Q_\infty(1-e^{-K_1 t})$ $\mathrm{Ln}(Q_\infty - Q_t) = \mathrm{Ln}Q_\infty - K_1 t$	K_1
Hixon–Crowell	$Q_i^{1/3} - Q_{solid}^{1/3} = K_s t$ $Q_\infty^{1/3} - (Q_\infty - Q_t)^{1/3} = K_s t$ $Q = Q_\infty - \left[\sqrt[3]{Q_\infty} - K_s t\right]^3$	K_s
Weibull	$Q_t = Q_\infty(1-e^{-(t/Td)^b})$	b: shape parameter T_d: time necessary to dissolve 63.2% of the dose When $b = 1$; first order
Higuchi	$Q_t = K_d\sqrt{t}$	K_d
Baker–Lonsdale	$\frac{3}{2}\left[1-\left(1-\frac{Q_t}{Q_\infty}\right)^{2/3}\right] - \frac{Q_t}{Q_\infty} = K_d t$	K_d
Korsmeyer–Peppas	$\frac{Q_t}{Q_\infty} = K_k t^n$	K_k
Gompertz	$Q_t = Q_\infty e^{-e^{-((t-a)K)}}$	K, a
Hopfenberg	$\frac{Q_t}{Q_\infty} = 1-\left[1-\frac{K_0 t}{C_0 a_a}\right]^n$ C_0: uniform initial concentration of drug in the matrix; a_0: initial radius for a sphere or cylinder or the half-thickness for a slab; $n = 1$ for a slab, $n = 2$ for a cylinder, $n = 3$ for a sphere	K_0: erosion rate constant

Notes: Q_t, amount of drug dissolved; Q_0, amount dissolved at time 0; Q_∞, amount dissolved when $t \to \infty$; Q_i, initial amount in the dosage form (dose); Q_{solid}, amount remaining in the dosage form; K_x, dissolution rate constant. When dissolution is complete and in the absence of lag time, in general, $Q_i = \text{dose} = Q_\infty$; Q_∞ can be treated as a parameter or fixed to the dose value or to 100% if the dependent variable is expressed as percentage dissolved.

dissolved from all the size fractions, which is used to calculate the dissolution gradient.

This equation was further modified (to Equation 4.3.35) to account for a time-dependent diffusion layer and for non-spherical geometries (Lu *et al.*, 1993).

$$-\frac{dQ^{i}_{solid}}{dt} = \frac{3(Q^{i}_{0})^{2/3}(Q^{i}_{solid})^{1/3}}{\rho(r^{i}_{0})^{2}}\left(C_{s} - \frac{Q^{T}_{diss}}{V}\right) \qquad (4.3.35)$$

Equation 4.3.35 is applied to small particles (<30 μm), whereas for particles bigger than 30 μm, the diffusion layer thickness is fixed to a value of 30 μm. This model was successfully applied to performing simulations of plasma profiles, by varying the absorption rate constant and dose/solubility ratio to explore in which scenarios the change in particle size leads to relevant changes in the maximum dose absorbed (Johnson and Swindell, 1996).

A similar approach to the dissolution process from a mechanistic point of view was used by de Almeida *et al.* (1997). They developed a Fortran subroutine which accounts for both the reduction in the number of particles as dissolution proceeds and the polydisperse nature of the powder. They applied the model to the dissolution of ibuprofen powders with different average diameters and their mixtures, and found a critical diameter of 22 μm and a proportionality factor between particle diameter and boundary layer thickness of 0.26, indicating that for particles less than 22 μm in diameter, the boundary layer thickness is approximately half the radius.

A review, including practical applications of other advanced methodologies such as Monte Carlo simulations or the fractal kinetics concept, can be found in Dokoumetzidis *et al.* (2005). There are several recent examples of the application of stochastic models used to describe the dissolution process (Lansky and Weiss, 1999, 2001, 2003; Lansky *et al.*, 2004; Schreiner *et al.*, 2005).

Modelling dissolution in the context of modelling release processes from the controlled-release dosage form can help to identify the controlling steps in the process and thereby to simulate the effects of the device parameters on the resulting release profile. Some recent reviews of these models are available in the following references: Grassi and Grassi (2005) and Siepmann and Peppas (2001) for a general overview; Grassi and Grassi (2005) for matrix devices and bio-erodible systems; Kanjickal and Lopina (2004) for polymeric systems; and Craig (2002) for solid dispersions of water-soluble polymers.

4.3.3.3 *In vitro–in vivo* correlations

An *in vitro–in vivo* correlation (IVIVC) is a 'predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response' (FDA, Center for Drug Evaluation and Research, 1997). The *in vitro* property is generally the rate or extent of drug dissolution or release, while the *in vivo* response is the plasma drug concentration or amount of drug absorbed. The main objective of developing and evaluating an IVIVC is to establish the *in vitro* dissolution test as a surrogate for human bioequivalence studies. There are several levels of IVIVC (see the FDA guidance document (1997) for more information) but the most informative one is level A correlation.

A level A correlation represents a point-to-point relationship between *in vitro* dissolution and *in vivo* input rate. In a linear correlation, the *in vitro* dissolution and *in vivo* input curves such as absorption rate may be directly superimposable or may be made to be superimposable by the use of a simple scaling factor. Non-linear correlations, while uncommon, may also be appropriate. Whatever model is used to establish a level A IVIVC, it should predict the *in vivo* plasma concentrations from the applied *in vitro* data.

Level A correlation is the only IVIVC that regulatory authorities allow for the prediction of plasma concentration profiles. The development of a level A IVIVC can be performed with two different approaches that are commonly described as the 'one-step' and 'two-steps' methods, respectively. In both cases it is necessary to develop formulations with different release rates such as slow, medium and fast. The *in vitro* dissolution profiles, as well the *in vivo* plasma concentration profiles in healthy human volunteers, have to be obtained for all these formulations. It is also necessary in the first step to obtain an *in vivo* 'absorption' or *in vivo* 'dissolution' profile from pharmacokinetic data. The second step consists of establishing the link, i.e. to modulate the link between *in vitro* and *in vivo* profiles and, finally, to validate the model, i.e. validate the prediction of the plasma levels from *in vitro* data.

4.3.3.3.1 Obtaining the absorbed fraction from plasma profiles

This process is schematised in Figure 4.3.7. Once the *in vitro* (dissolution) and *in vivo* concentration profiles (plasma levels) have been obtained, in order to establish the correlation between them the first problem to be solved is how to calculate the *in vivo* amounts *absorbed* at each time

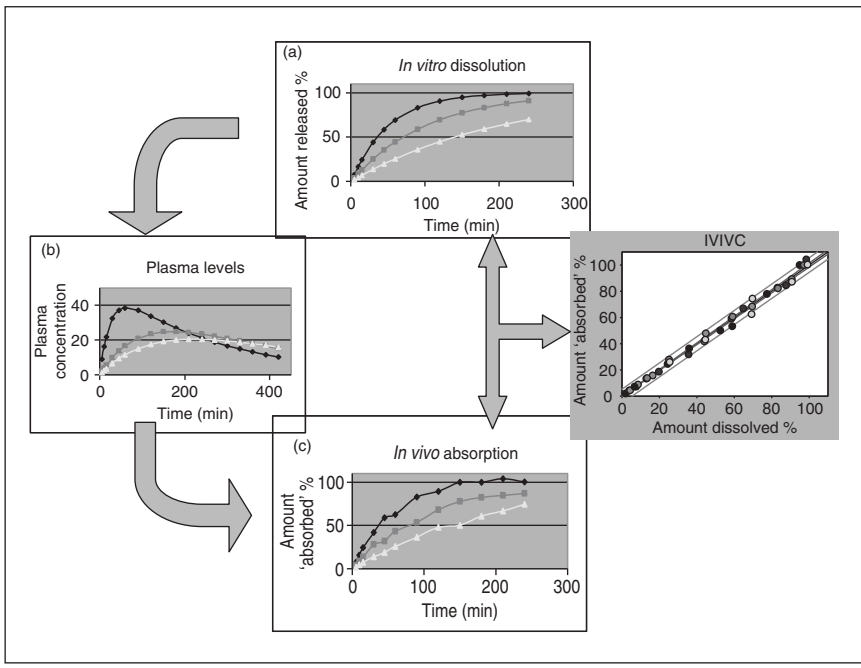


Figure 4.3.7 Scheme of the development an *in vitro–in vivo* correlation in two steps: (a) dissolution profiles of formulations with different release rates, i.e. slow, medium, fast; (b) plasma profiles obtained in human volunteers after administration of the slow-, medium- and fast-release formulations; (c) amounts`absorbed' versus time, calculated from the plasma levels (see methods in the text). The second step consists of establishing the link function between *in vitro* profiles (a) and *in vivo* profiles (c).

point, from the plasma profiles. At this point, it is convenient to remember the definition of fraction absorbed. Drug absorption must be distinguished from systemic availability. The systemic availability refers to the fraction of dose reaching the systemic circulation. Systemic availability F_{sys} can also be expressed by Equation 4.3.36:

$$F_{sys} = f_a{}^*(1-E_g)^*(1-E_h) \tag{4.3.36}$$

where f_a is the fraction absorbed, E_g the gut extraction ratio and E_h the hepatic extraction ratio. Absorption of a drug substance refers to its process of crossing the apical membrane of the enterocytes. So, the rate and extent of drug absorption may be considered simply as the rate and extent of drug permeating the apical membrane of the enterocytes. This section describes how the absorbed fraction f_a may be calculated from a plasma concentration profile, but the reader should bear in mind that in many

books and papers, F_{sys}, i.e. the fraction reaching the systemic circulation or available fraction, is misnamed and called fraction *absorbed*.

The mathematical methods for calculating the oral fraction absorbed are classified as model dependent or model independent. Model-dependent methods assume a particular compartmental pharmacokinetic model for the drug disposition, while the more general deconvolution methods are considered model independent as there is no need for assuming any particular pharmacokinetic model. Wagner–Nelson and Loo–Riegelman are examples of model-dependent methods:

The Wagner–Nelson method can be applied to one-compartment drugs. It is, essentially, a mass balance calculation. All the drug that has been absorbed up to a time point Q_{at}, is either in the body, Q_{ct}, or has been already eliminated, Q_{et}. The equations and an example of the Wagner–Nelson plot are illustrated in Figure 4.3.8. From the

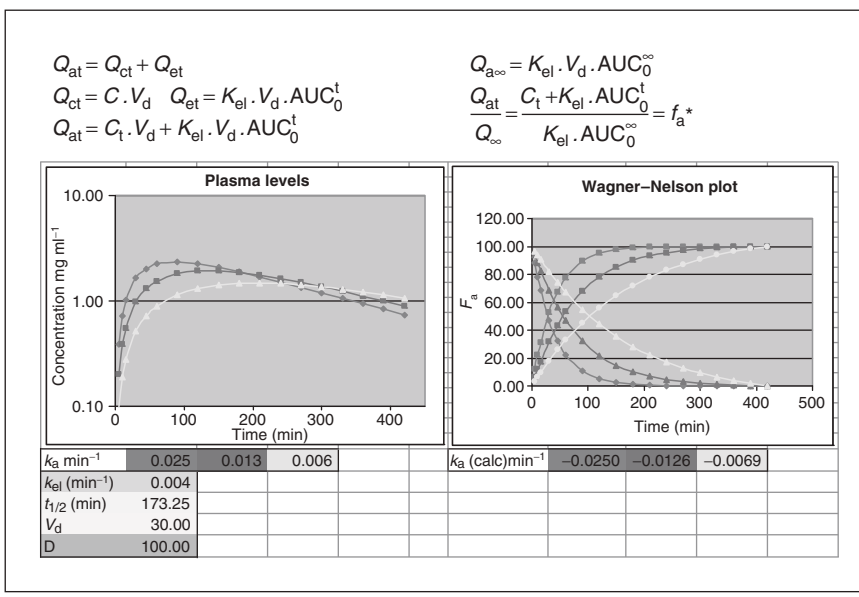


Figure 4.3.8 Wagner–Nelson mass balance. At any time all the drug that has been absorbed is either in the body or has been already eliminated. The plasma levels (on the left) have been simulated for a one-compartment drug with different absorption rate constants. On the right the Wagner–Nelson plots and the remaining concentrations on the absorption site from which the absorption rate constant can be estimated. Q_{at} = amount absorbed at time t; Q_{ct} = amount in the body at time t; Q_{et} = amount eliminated at time t; $Q_{a\infty}$ = total amount absorbed; V_d = distribution volume; C_t = plasma concentration at time t; k_{el} = elimination rate constant; AUC = area under the curve; f_a^*:fraction of the bioavailable dose.

plasma concentrations and the area under the curve (AUC calculated by the trapezoidal rule), and the elimination rate constant, k_{el} (obtained from the terminal slope), it is possible to obtain f_a at each time. In this case, f_a represents the fraction of the bioavailable dose that has already been absorbed at time t. Wagner–Nelson plots will always reach 100% even if bioavailability is not complete, because the fraction of bioavailable amount is the parameter calculated and not the fraction of the dose. On the other hand, in general, it is possible to perform Wagner–Nelson calculations without having intravenous data, as the elimination rate constant is estimated from the terminal slope of the plasma profiles. This latter approach is, of course, based on the assumption that the absorption is not influencing elimination (flip-flop).

In the case of a flip-flop, in which the decline of plasma concentration is absorption-rate limited, this leads to inaccurate estimation of the absorption rate constant; this could happen when evaluating controlled-release products.

A second problem that could arise when an intravenous reference is not available is the misidentification of the kinetic model of the drug. The results of Wagner–Nelson analysis are valid only if the one-compartment model represents the disposition kinetics. For two-compartment drugs it is necessary to account for the amount of drug in the peripheral compartment.

The Loo–Riegelman mass balance is similar to the Wagner–Nelson one. The problem now is the calculation of the amount and concentration of drug in the peripheral compartment. The equations summarised in Figure 4.3.9 are the exact solution of the Loo–Riegelman equation (a), published by Wagner, and an approximate solution. Both are easily implemented in Excel sheets. The approximate solution can be applied if the plasma concentrations have been obtained in short time intervals and if the change in concentration between two consecutive time points is approximated by a linear function. As can be seen, for these calculations the disposition parameters obtained from intravenous administration of the drug are necessary. Wagner–Nelson and Loo–Riegelman are model-dependent methods but in fact could also be considered as special cases of the more general deconvolution methods.

Deconvolution does not need to assume any kinetic model for drug disposition. Convolution can be applied to linear systems or, in other words, the convolution integral is the mathematical definition of a linear system.

The convolution principle may be expressed as follows:

$$C_{(t)} = C_{(\delta)} * f_{(t)} \tag{4.3.37}$$

The response function, $C_{(t)}$, is obtained by convolution of the unit impulse response function, $C_{(\delta)}$, with the input function, $f_{(t)}$. Mathematical convolution has been functionally expressed here by the asterisk. The fundamentals of this theory and the application in the pharmacokinetic field were set up in the 1980s (Veng-Pedersen, 1980a,b; Iga *et al.*, 1986; Veng-Pedersen and Miller, 1987), and there are many recent examples of their use (Gillespie and Veng-Pedersen, 1985; Modi *et al.*, 2000; Sirisuth *et al.*, 2002; Dutta *et al.*, 2005).

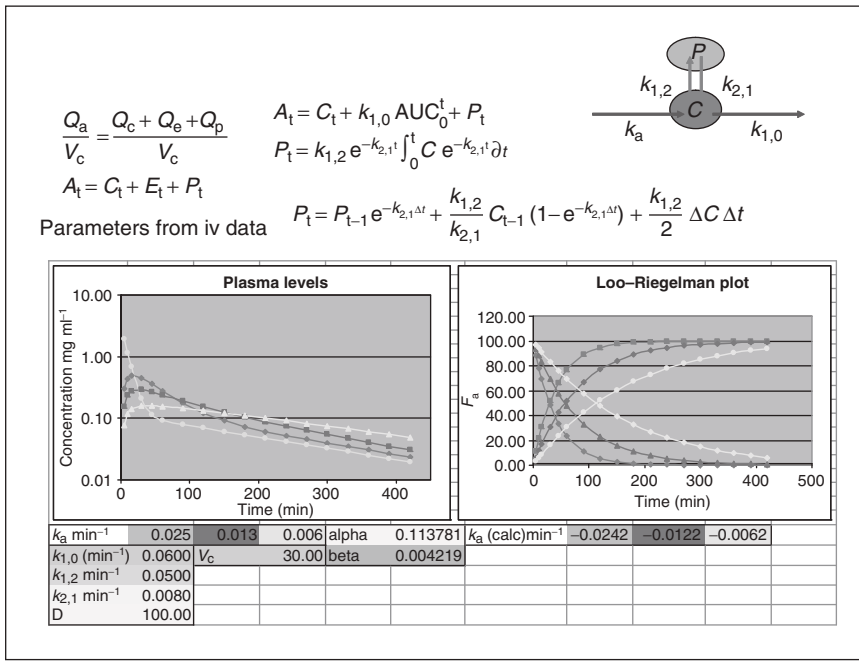


Figure 4.3.9 Loo–Riegelman mass balance. At any time all the drug that has been absorbed is either in the central compartment or in the peripheral compartment, or it has already been eliminated. The plasma levels (on the left) have been simulated for a two-compartment drug with different absorption rate constants. On the right the Loo–Riegelman plots and the remaining concentrations on the absorption site from which the absorption rate constant can be estimated are shown. Q_a = amount absorbed; Q_c = amount in the central compartment; Q_p = amount in the peripheral compartment; Q_e = amount eliminated; the amounts are transformed in apparent concentrations (A, C, P, E) dividing by the V_c = central compartment volume; $k_{1,0}$ = elimination rate constant from central compartment; $k_{1,2}$, $k_{2,1}$ = distribution rate constants.

Under conditions of linearity and time invariance, the transport of drug from the absorption site to the plasma can be completely expressed by the three functions in Equation 4.3.37. $C_{(t)}$ refers to the concentration profile obtained when the drug is placed at the absorption site and the concentration is measured in plasma. $C_{(\delta)}$ is also known as the characteristic or weighting function. In practical terms, it represents the concentration–time profile after an intravenous bolus, divided by the dose. $f_{(t)}$ represents the transfer function that governs the movement of mass from the absorption site to the plasma. The knowledge of any two of these three functions allows determination of the third one. Deconvolution is the mathematical inverse of convolution. This refers to the situation where a knowledge of $C_{(t)}$ and $C_{(\delta)}$ is used to obtain the input function, $f_{(t)}$ (Pithavala *et al.*, 1997). There are different deconvolution techniques: analytic deconvolution using Laplace transforms (Purves, 1995; Schalla and Weiss, 1999), implicit deconvolution by curve fitting (Veng-Pedersen, 1980), numeric deconvolution methods such as the point-area method (Yu *et al.*, 1996; Yeh et al., 2001) among others. Analytic deconvolution and implicit deconvolution by curve fitting are described in Figures 4.3.10 and 4.3.11.

The Wagner–Nelson and Loo–Riegelman methods lead to calculation of the bioavailable fraction. In the deconvolution–convolution method, the *in vivo* input function that is obtained depends on the reference used during the deconvolution process.

Implicit deconvolution by curve fitting

UIR $C_{(\delta)(t)} = \frac{1}{D}\left(\frac{D}{V_c} e^{-k_{el}t}\right)$

Response $C_{(t)} = \frac{F D k_a}{V_c (k_a - k_{el})} (e^{-k_{el}t} - e^{-k_a t})$

} Simultaneous fit

Input $f_{(t)} = F\ D\ k_a e^{-k_a t}$

Figure 4.3.10 Deconvolution by curve fitting implies the simultaneous curve fitting of the response function (i.e. the plasma curve after the oral administration) and the unit impulse response UIR (i.e. the response after a bolus divided by the dose). Once the disposition parameters are obtained, it is possible to extract the parameters of the input function.

Analytic deconvolution : Laplace transforms

$$c(t) = \int_0^t f(\tau)\, c_\delta(t-\tau)\, d\tau$$

Response Input Unit impulse response

$$f(t) = F\,D\,k_a e^{-k_a t} \rightarrow \ell[(f)t] = \frac{FDk_a}{(s+k_a)} \quad \text{input} \quad \text{Step 1}$$

$$c_\delta(t) = \frac{1}{D}\left(\frac{D}{V_d} e^{-k_{el} t}\right) \rightarrow \ell[c_\delta(t)] = \frac{1}{V_d(s+k_{el})} \quad \text{Unit impulse response}$$

$$\ell[f(t)]\ell[c_\delta(t)] = \frac{FDk_a}{V_d(s+k_a)(s+k_{el})} \quad \text{Step 2}$$

$$\ell^{-1}\left[\frac{FDk_a}{V_d(s+k_a)(s+k_{el})}\right] = \frac{FDk_a}{V_d(k_a - k_{el})}\left(e^{-k_{el} t} - e^{-k_a t}\right) \quad \text{Step 3}$$

Figure 4.3.11 Steps to perform analytic convolution and deconvolution by using Laplace transforms. To convolve two functions: (1) take the Laplace transform of each one; (2) multiply the transformed functions; and (3) take the inverse transform.

If an intravenous bolus is used as a reference administration, the unit impulse response of the system corresponds to the disposition of the drug, and thus the input function incorporates all the previous processes, that is, dissolution, absorption and first pass.

In the case of using an oral solution as a reference, the unit impulse response already includes absorption and first pass besides disposition, so, by deconvolution, the input function that is estimated corresponds to the release or dissolution rate.

If the reference is an immediate release (IR) dosage form of the same drug, the unit impulse response incorporates the dissolution from the IR dosage form, and thus the input represents the release rate from the modified-release dosage form.

4.3.3.3.2 Establishing the link function

With the methods described in the previous section, the input profiles (i.e. fraction absorbed versus time) are estimated from the plasma levels. The *in vitro* dissolution profiles have to be characterised, ideally, using the same time-sampling scheme and with a dissolution medium mimicking the *in vivo* dissolution conditions. The next step is to establish the correlation between the fraction absorbed and fraction dissolved at

similar time intervals. When dissolution *in vivo* is the limiting step for absorption, and the *in vitro* dissolution test reflects *in vivo* conditions, the *in vivo* input profiles and the *in vitro* dissolution profiles are superimposable, and then the link function between *in vitro* and *in vivo* fractions (for all three formulations) is a single linear correlation. In the second step, plasma concentrations are predicted from the *in vitro* dissolution data using the link model and by convoluting the unit impulse response characterised in the first step.

A one-step method involves simultaneous fitting of the *in vitro* and *in vivo* data, to obtain the parameters of the link function. Then, it is possible to compare directly the plasma concentrations predicted from the model and those observed. As there is no deconvolution step, the procedure does not require the reference administration (Buchwald, 2003).

Validation of the model consists of estimating the magnitude of error in predicting *in vivo* bioavailability, which is the ability of the model to predict C_{max} and AUC from the IVIVC model and the dissolution profiles. The internal predictability correspond to the ability to predict C_{max} and AUC from the data sets that have been used in the development of the correlations, whereas the external predictability refers to the ability of predictions for a data set not used in the development of the correlation.

The definition of a level A correlation, i.e. 'the *in vitro* and *in vivo* dissolution curves are superimposable', relates to the identity model. When these curves are not superimposable there are two possible strategies that may be adopted.

The first is to change the *in vitro* dissolution conditions in order to find conditions required for the curves to be superimposable. This strategy corresponds to the use and development of the so-called biorelevant dissolution media (Dressman and Reppas, 2000; Nicolaides *et al.*, 2001; Vertzoni *et al.*, 2004) that are intended to simulate the main *in vivo* physiological variables such as pH of luminal fluids, the presence of surfactants and the volume available for dissolution.

The second approach is to find an alternative non-linear model that describes the relationship between the two curves or to include scaling factors, for either the time or the amplitude. Dunne and collaborators developed several non-linear mathematical models to obtain IVIVCs (Dunne *et al.*, 1997, 1999; O'Hara *et al.*, 2001). For the derivation of their equations, the authors considered the time at which a molecule goes into solution (either *in vivo* or *in vitro*) as a random variable (see Figure 4.3.12). $F_{(t)}$ represents the distribution function of the random variables. The practical meaning of the distribution function is the fraction of dose dissolved from each unit at time *t* under *in vitro* or *in vivo* conditions.

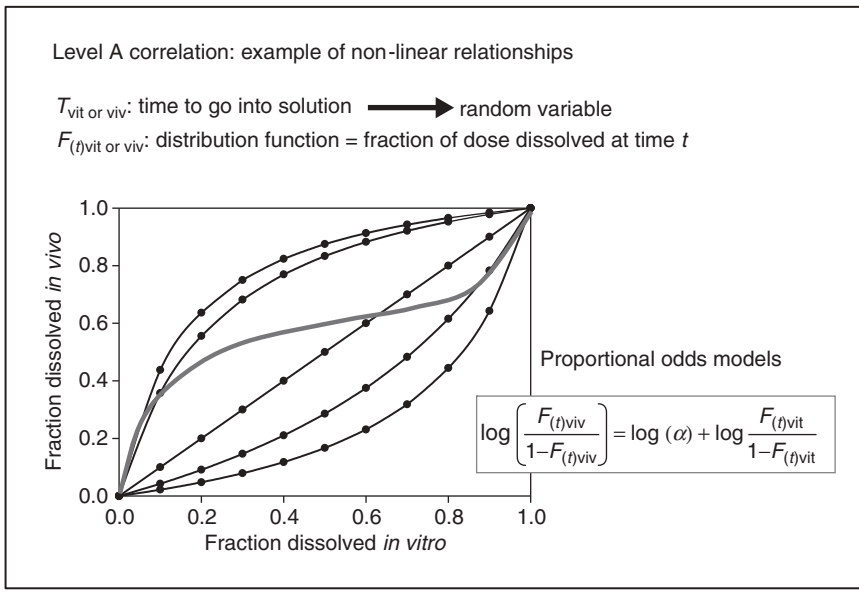


Figure 4.3.12 Example of a non-linear IVIVC relationship, the proportional odds model from Dunne *et al.* (1997). The time at which a molecule goes into solution either *in vivo* or *in vitro* is a random variable. $F_{(t)}$ represents the distribution function of the random variable or, in practical terms, the fraction of dose dissolved from each unit at time t under *in vitro* or *in vivo* conditions. The odds function expresses the ratio of the probability that a molecule will enter into solution prior to time t to the probability that it will not. The proportional odds model states that at any time the odds function *in vivo* is proportional to the corresponding function *in vivo*. α is the proportionality constant. When the proportionality constant equals one, the odds model collapse to the identity or linear model. Adapted from Dunne *et al.* (1997).

The odds function expresses the probability ratio of a molecule entering into solution or not, prior to time t. The proportional odds model states that at any time the odds *in vivo* function is proportional to the corresponding *in vitro* function; this may be described by Equation 4.3.38:

$$\left(\frac{F_{(t)\text{viv}}}{1-F_{(t)\text{viv}}}\right) = \alpha\left(\frac{F_{(t)\text{vit}}}{1-F_{(t)\text{vit}}}\right) \tag{4.3.38}$$

and in logarithmic expression by Equation 4.3.39:

$$\log\left(\frac{F_{(t)\text{viv}}}{1-F_{(t)\text{viv}}}\right) = \log(\alpha) + \log\left(\frac{F_{(t)\text{vit}}}{1-F_{(t)\text{vit}}}\right) \tag{4.3.39}$$

where α is the proportionality constant. When the proportionality constant equals one, the odds model collapses to the identity or linear model.

Figure 4.3.12 represents a different relationship between the fraction of drug dissolved *in vivo* and *in vitro* for a range of values of α from 0.2 to 7. The model increases its flexibility when the parameter α change with time, but in these cases the relationship between the *in vivo* and the *in vitro* odds functions is no longer described by one of the curves in Figure 4.3.12 but by a shifted curve (grey line). One possible rationale to allow for α changing with time is when there may be observed changes within the environment such as pH changes within GI fluids as the dosage forms proceeds along the GI tract.

Another example of a non-linear IVIVC model described in the literature is the one developed by Polli *et al.* (1996). The equations are based on the following mass balance equation:

$$M_a = M_o - (M_{ff} + M_{gi}) \tag{4.3.40}$$

where M_a is the amount absorbed at time t, M_o represents the dose (initial amount of drug in the dosage form), M_{ff} is mass of drug still in the dosage form and M_{gi} the mass of drug in solution in GI fluids. The final equation is:

$$F_a = \frac{1}{f_a}\left(1 - \frac{\alpha}{\alpha - 1}(1 - F_d) + \frac{1}{\alpha - 1}(1 - F_d)^{\alpha}\right) \tag{4.3.41}$$

where F_a is the fraction of the total amount of drug absorbed at time t, f_a is the fraction of the dose absorbed at $t = \infty$, α is the ratio of the apparent first-order permeation rate constant (K_{papp}) to the first-order dissolution rate constant (K_d), and F_d is the fraction of drug dose dissolved at time t. For high α values, i.e. when the absorption process is limited by the dissolution, the correlation is linear. But for lower α values, when absorption is the rate-limiting step the correlation shows an 'L'-shape as can be seen in Figure 4.3.13. This would be the most usual situation for IR products of drug substances with high solubility and dissolution rate. This information is useful for identifying the limiting steps in the absorption process in new formulations. Drug products with high α values would be those needing special attention in their technological variables, as any change in their dissolution profile will have a clear impact on their absorption rate. On the other hand, products with low α values indicate that the rate-limiting step is membrane permeation and, thus, the absorbed fraction is not so sensitive to any modification in the dissolution of the product.

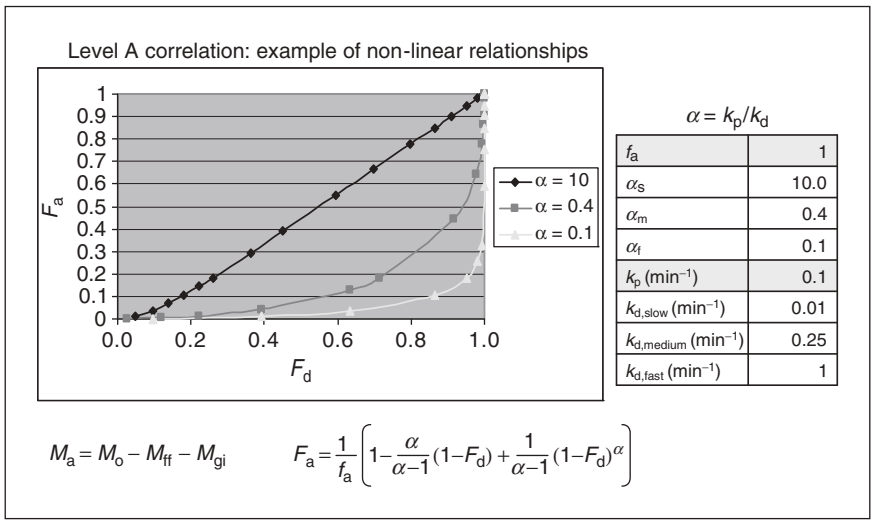


Figure 4.3.13 Example of non-linear IVIVC. Model of Polli *et al.* (1996). The model is based on the mass balance: M_o is the dose (initial amount of drug in the dosage form); M_{ff} the mass of drug remaining in the dosage form; M_{gi} the mass of drug in solution in the GI fluids; α represents the ratio between the permeation rate constant (k_p) and the dissolution rate constant (k_d). For explanation see text.

Once the IVIVC model has been adequately validated, the *in vitro* release profiles can be used to simulate the expected *in vivo* profiles and then to set the dissolution specifications that will ensure *in vivo* bioequivalence. A very useful and illustrative series of papers showing the basic concepts in IVIVC and how to program the calculation in Excel worksheets has been published by Langenbucher (2002, 2003a, b, 2005).

4.3.4 Conclusions

A good modelling/biosimulation approach can be a useful tool in preclinical investigations and dosage form development, and there are many examples described in the literature that could help the reader to understand the advantages of using these simulations in terms of process optimisation, speeding development and maximising the information that can be extracted from large amounts of data. The following four examples have been selected from the literature for their relative simplicity, to show how to answer common questions in drug delivery, for instance, how to use modelling as a tool to explore the release mechanism from modified-release formulations (Cox *et al.*, 1999; Fukui *et al.*, 2002; Lutchman *et al.*, 2005), the use of simulation to explore the relevance

of differences in release rate on the *in vivo* exposure (Morita *et al.*, 2000; Yu *et al.*, 2001), how the release profile should be designed to achieve and maintain the target therapeutic plasma levels (Mrhar *et al.*, 1999; Morita *et al.*, 2000), or how to use simulations as tools for preclinical investigations and formulation development (Dannenfelser *et al.*, 2004, Kuentz *et al.*, 2006).

In many of these examples, specialised software is used to implement the models or simulations. In Appendix 4.3.2, a table with modelling and simulation software is included. The list has been adapted and updated from Rowland *et al.* (2004).

From the parameters obtained after model-fitting procedures, it is possible to perform deterministic simulations, i.e. without any noise or error in the predicted data. Of course, this is not the best representation of the reality, and even if this approach could be useful for understanding the system, the best-case situation would be to incorporate the real-life variability in the simulations. The non-linear mixed effect models (commonly used in the population pharmacokinetic field) include the so-called 'fixed effects' (the structural parameters) and 'random effects' (the variability parameters). Variability parameters are used to account for the possible sources of variation; these could be inter-subject variations, inter-occasion variability, and residual variability. These same concepts could be transferred to the dosage form evaluation field and the reader can find the general principles in specialised pharmacokinetic literature. Some recent references are Krishna (2004) and Bonate (2005).

Appendix 4.3.1: Objective functions

Least squares

In a fitting procedure we are looking for the set of parameters that produces predicted values of the dependent variable that are as close as possible to the experimental ones. The mathematical expression of this concept is the least square objective function (OF):

$$\mathrm{OF} = \sum_{i=1}^{n} (y_{\mathrm{exp}} - y_{\mathrm{pred}})^2 \qquad (4.3.42)$$

where y_{exp} represents the experimental data and y_{pred} refers to the values predicted by the model, giving a particular value for the parameters. Linear and non-linear regression by least squares means finding the combination of parameters that minimises this objective function.

Weighted least squares

Even if the errors in each data point are independent and normally distributed, the magnitude of the error or variance may be different. When the magnitude of the errors in the dependent variable is high (as it is usual with biological data) and/or when the range covered is very wide (more than one order of magnitude), then an adequate weighting scheme becomes necessary:

$$\mathrm{OF} = \sum_{i=1}^{n} w_i \left(y_{\mathrm{exp}} - y_{\mathrm{pred}}\right)^2 \tag{4.3.43}$$

where w_{i} are the weights assigned to each residual value.

For instance, when the magnitude of the error is proportional to the magnitude of the dependent variable in the experimental range (so the coefficient of variation (CV) in the dependent variable is constant), then the usual weight is:

$$w_{\mathrm{i}} = \frac{1}{\left(y_{\mathrm{exp}}\right)^2} \tag{4.3.44}$$

In Equation 4.3.44, if, instead of using the experimental data points the predicted ones are used, the method is called iteratively reweighted least squares, as the weights are changed in each iteration step.

Extended least squares

In this method, a variance equation is included in the objective function expression and the parameters of the variance equation are estimated simultaneously with the model parameters.

$$\mathrm{Variance} = \mathrm{f}(P,\ Pv,\ Px) \tag{4.3.45}$$

$$y_{\mathrm{pred}} = \mathrm{f}(P,\ x) \tag{4.3.46}$$

$$\mathrm{OF} = \sum_{i=1}^{i=n} \frac{\left(y_{\mathrm{exp}} - \mathrm{f}(P,x)\right)^2}{(\mathrm{variance},\ \mathrm{f}(P,Pv,x))} + \mathrm{Ln}(\mathrm{variance},\ \mathrm{f}(P,Pv,x)) \tag{4.3.47}$$

where P are the model parameters and Pv the variance parameters.

The second term in the objective function is a penalty term included in order to avoid the objective function becoming smaller due to the variance term becoming high regardless of the fit of the data.

More definitions of objective functions and a good review of linear and non-linear regression concepts applied to pharmacokinetics and biopharmaceutics can be found in Bourne's books (Bourne, 1995; Bourne *et al.*, 1986). Some basic principles are summarised in the next section.

Appendix 4.3.2: How does non-linear regression work?

In linear regression, finding the solution of the objective function is quite straightforward. The process consists of obtaining the partial derivatives of the objective function (sum of squared residuals) with respect to the intercept and the slope (the line parameters) and equalling them to zero. The system of equations that is obtained has an algebraic solution (see Appendix 4.3.1). When the model and the equation are not linear, in general, the system of equations obtained after derivation of the objective function is not linear and does not have an algebraic solution. To find the solution it is necessary to use an iteration procedure, as well as to define the so-called initial estimates, as a starting point for the search of the parameters that minimise the objective function.

The majority of the searching algorithms or iteration methods can be classified as gradient-based methods or numerical methods. An example of gradient methods is the Gauss–Newton algorithm that is based on the Taylor's series linear approximation to the objective function.

Let us consider the Taylor's approximation (taking just the two first terms) to a non-linear function that it is being evaluated for a set of initial parameters P^0:

$$y_i = f(x_i, P) = f(x_i, P^0) + \sum_{j=1}^{j} \left[\frac{\partial f(x_i, P)}{\partial P_j}\right]_{P=P^0} (P - P^0) \qquad (4.3.48)$$

This expression is analogous to this one:

$$y = f(a) + (x - a)f'(a) \qquad (4.3.49)$$

Reorganising the terms:

$$y_i - f(x_i, P^0) = \sum_{j=1}^{j} \left[\frac{\partial f(x_i, P)}{\partial P_j}\right]_{P=P^0} (P - P^0) \qquad (4.3.50)$$

The equation can be rewritten this way:

$$w = V_{i,j} * B \qquad (4.3.51)$$

where w are the differences, $y_i - f(x_i, P^0)$, B is the vector of parameters $(P - P^0)$, and $V_{i,j}$ the matrix of partial first derivatives (called the Jacobian matrix). This matrix has as many columns as parameters and the number of rows corresponds to the number of data points.

This last equation is analogous to the linear regression equation (in matrix notation) $y = x^* B$

Thus, the same solution can be applied, and the vector of parameters B is:

$$B = (V_{i,j}^T \, V_{i,j})^{-1} \, V_{i,j} \, w \tag{4.3.52}$$

(see Appendix 4.3.1).

From B, a new vector of parameters P^1 is obtained. Then, the same calculation can be done again, that is:

$$P_{j+1} = P_j + (V_{i,j}^T \, V_{i,j})^{-1} \, V_{i,j} \, w \tag{4.3.53}$$

An example of the first step of the iterative process based on the Gauss–Newton algorithm is summarised in Table 4.3.5, taking the Michaelis–Menten equation as an example. The example is adapted from a similar one in Endrenyi's book (Endrenyi, 1981). This *one-step* calculation is repeated many times in the standard non-linear regression software until a convergence criterion is achieved. In non-linear regression, a starting point (initial estimates) is necessary, as well as a criterion to stop the search. In general, the convergence criterion is defined in terms of the relative improvement on the sum of squares (the objective function) with equations similar to Equation 4.3.54:

$$|(SS_{n+1} - SS_n)/SS_n| < \delta \tag{4.3.54}$$

where δ is the convergence criterion and SS_{n+1} and SS_n the sum of squares obtained in the $n+1$ and n iterations.

The Nelder–Mead algorithm (or simplex method) (Nelder and Mead, 1965; Walters, 1991) is a numerical method based on the construction of a figure with $p+1$ vertices (where p is the number of parameters). In a problem with two parameters, the simplex would have three vertices. Each of these vertices represents a combination of values of P_1 and P_2 at which the objective function is evaluated. The point with the highest value for the objective function is projected over the simplex centroid, thus obtaining a new simplex figure and restarting the process again. A graphical representation of this procedure is shown in Figure 4.3.14. The lines represent combinations of parameter values rendering the same objective function value (in a similar fashion to pressure isobars).

Table 4.3.5 Example of first iteration

$x = concentration$	$y_{exp} = rate$	y_{pred}	$Residual = (y_{exp} - y_{pred})$	$Residual^2$	$dy/dK_m = C/(K_m + C)$	$dy/dV_m = -V_m C/(K_m + C)^2$
0.010	0.01960	0.01275	0.00685	4.69×10^{-5}	0.01961	−0.02499
0.030	0.05628	0.03679	0.01949	3.80×10^{-5}	0.05660	−0.06942
0.070	0.10948	0.07982	0.02965	8.79×10^{-5}	0.12281	−0.14004
0.100	0.16205	0.10833	0.05372	2.89×10^{-5}	0.16667	−0.18056
0.300	0.32731	0.24375	0.08356	6.98×10^{-5}	0.37500	−0.30469
0.500	0.44547	0.32500	0.12047	1.45×10^{-5}	0.50000	−0.32500
1.000	0.57014	0.43333	0.13681	1.87×10^{-5}	0.66667	−0.28889
3.000	0.70972	0.55714	0.15258	3.33×10^{-5}	0.85714	−0.15918
5.000	0.73112	0.59091	0.14021	1.97×10^{-5}	0.90909	−0.10744
6.000	0.75360	0.60000	0.15360	2.36×10^{-5}	0.92308	−0.09231
8.000	0.70007	0.61176	0.08831	7.70×10^{-5}	0.94118	−0.07197
10.000	0.71021	0.61905	0.09116	8.31×10^{-5}	0.95238	−0.05896
			w	0.1270457	$V_{i,j}$	
				SS		

V_m	0.65	0.1151904	0.7651904			
K_m	0.5	−0.187224	0.3127757			
	P^0	$B = (P^1 - P^0)$	$P^1 = P^0 + B$			
$V_{i,j}$ transposed * $V_{i,j}$		Inverse ($V_{i,\ j}$ transposed * $V_{i,j}$)		SS/df		
5.087	−0.96427	0.36692	0.898826	0.0127046	0	
−0.96430	0.3936313	0.898834	0.7422832	0	0.0127046	
$V_{i,j}$ transposed * w		Inverse ($V_{i,j}$ transposed * $V_{i,j}$) * SS/df = covariance matrix				
0.76657		0.0046615	0.0114192	= Var(V_m)		
−0.1848		0.0114192	0.0602487	= Var(K_m)		
					EE(V_m)	0.0682752
					EE(K_m)	0.245456

Notes: EE, standard estimation error; df, degrees of freedom (number of points – number of parameters); Var, variance; V_m, maximal velocity; K_m, Michaelis–Menten constant; SS, sum of squared residuals; $V_{i,j}$, matrix of first partial derivatives; w, differences between experimental and predicted values; in order to further reduce SS and estimate the parameters the next step would consist of taking P^1 values and using them as new set of P^0 values. The iteration process continues until convergence criteria are achieved. See text for detailed explanation. Adapted from (Endrenyi, 1981)

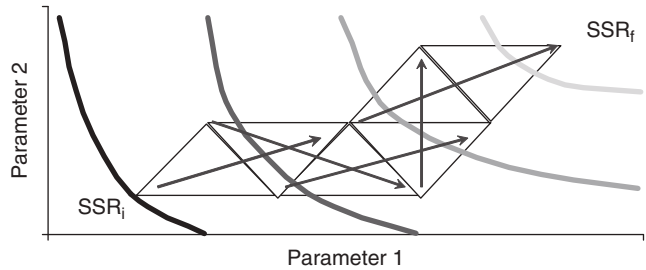


Figure 4.3.14 Graphical representation of Nelder–Mead algorithm (or simplex method) in a problem with two parameters. The simplex has three vertexes. Each of these vertexes represents a combination of values of P_1 and P_2 (parameters) at which the objective function is evaluated. The point (combination of P_1 and P_2) with the highest value for the objective function is projected over the simplex centroid to obtain the new simplex figure. The lines represent combinations of parameters values rendering the same objective function value. SSR_i, initial value of the sum of squared residuals; SSR_f, final value of the sum of squared residuals. $SSR_f << SSR_i$.

Understanding how the values of the parameters are estimated in non-linear regression is very useful in order to design experiments and optimise the information that could be obtained from the experimental data.

Table 4.3.5 shows how the standard error of estimation is calculated. In order to get the variance of the parameter, the covariance matrix is computed. The inverse of the covariance matrix $(V_{i,j}^{T}(\text{diag}\, w)V_{i,j})$ is the Fisher information matrix. Maximising the determinant of the Fisher information matrix will result in decreasing the variance of the parameter on the pre-specified model. In Figure 4.3.15, dy/dK_m is represented (one of the parameters of the example in Table 4.3.5) versus the concentration (x) and its squared value. In order reduce the estimation error, it is advisable to include in the experimental design those values of the independent variable at which dy/dK_m is higher (in absolute value), in order to make the variance–covariance matrix smaller. In summary, the estimation error will depend on the experimental design (as this impacts the amount of information about the parameters that is included in the data $(V_{i,j})$) and on the residual variability. The residual error includes the pure error, as this could be the analytical error, random variability and the potential lack of fit (i.e. discrepancies between the experimental and predicted values if the model is not the correct one) (Franz *et al.*, 1996; Flaherty *et al.*, 2006).

There are various forms of optimality criteria that are used to select the points for a design: one popular criterion is *D-optimality*, which seeks to maximise $|V_{i,j}^{T}(\text{diag}\, w)V_{i,j}|$, the determinant of the *information*

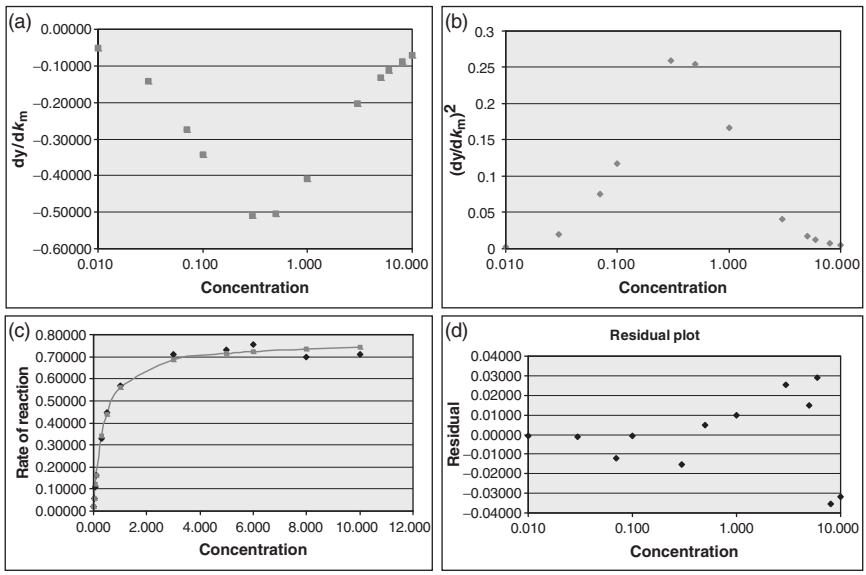


Figure 4.3.15 (a) Derivate of the dependent variable with respect to the parameter (K_m). (b) Squared value of the derivate in panel a. (c) plot of the dependent variable (rate of reaction) versus the independent one (substrate concentration). (d) Residual plot: differences between the experimental and the predicted values (residuals) versus concentration (independent variable). For explanation see text.

$matrix V_{i,j}^{T}(\mathrm{diag}\, w) V_{i,j}$ of the design. This criterion minimises the generalised variance of the parameter estimates based on a prespecified model (Croarkin *et al*., 2005).

How well does the model fit the data?

Once the convergence criterion is achieved, it is useful to perform the fit again, by changing the initial estimates in order to check that the algorithm has found the true minimum instead of a local minimum. A second alternative is to perform the fit again but using a different iteration algorithm, if the software application allows doing so. It is advisable, besides the latter steps, to evaluate the sum of the squared residuals, and to inspect the graph with the experimental values and the fitted ones, to check how close the predicted curve is to the real data. On the other hand, the parameter values have to be evaluated, bearing in mind the assumptions of the model and their plausibility in the framework of the experiment. If the estimation errors of the parameters are high, that could indicate a bad experimental design or over-parameterisation of the model. This latter problem could be difficult to solve, as the range of

independent variable values could be restricted by other experimental constraints, and it is not always possible to remove a parameter from a non-linear model. Other criteria to judge the goodness of the fit are briefly discussed next.

Residual plot

Provided that the residuals have a mean of zero and constant variance, are independent, and follow a normal distribution, the plot of the residual versus the independent variable should appear as randomly scattered around the zero value. Any trend in the residual plot indicates either that the variance is not constant or that the model is inadequate.

Coefficient of correlation R

This indicates how closely a best-fit curve matches the given data and is also a measure of the joint variation of the dependent and the independent variable (adimensional covariance).

$$R = \frac{\sum_{i=1}^{n}(x_i - \bar{x})\,(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2 \sum_{i=1}^{n}(y_i - \bar{y})^2}} \tag{4.3.55}$$

Coefficient of determination R^2

The total variation of the dependent variable y around its mean can be explained in part by the model (the regression equation) and by random variation. In mathematical terms:

$$\mathrm{SC_{total}} = \mathrm{SS_{model}} + \mathrm{SS_{residual}} \tag{4.3.56}$$

$$\sum (y_i - \bar{y})^2 = \sum (\hat{y} - \bar{y})^2 + \sum (y_i - \hat{y})^2 \tag{4.3.57}$$

where $\hat{y}$ are the predicted values; y_i the experimental values and $\bar{y}$ is the dependent variable mean.

From this equation it is possible to estimate how much of the variability of the dependent variable is explained by the model, defining R^2 as:

$$R^2 = \frac{\mathrm{SS_{model}}}{\mathrm{SS_{Total}}} \tag{4.3.58}$$

The model is better at predicting the experimental data as this value approaches 1.

Comparing models of different complexity

Akaike information criterion (AIC)

This is a model-order selection criterion based on parsimony. More complicated models are penalised for the inclusion of additional parameters. The idea behind this parameter is that more complicated models with a large number of parameters would lead to a better fit but with fewer degrees of freedom and with less practical utility, so the criterion tries to find a balance between goodness of fit and model complexity. AIC is computed from the sum of squared residuals, and includes a penalising term for models with more parameters (P):

$$\mathrm{AIC} = \ln \mathrm{SS}_{\mathrm{res}} + 2P \tag{4.3.59}$$

The best model is the one with a lower AIC value

Snedecor's F test

Snedecor's F test (or Fisher ratio) is used to compare the residual variances of models of increasing complexity (sum of squares divided by their degrees of freedom). To establish if the decrease in the sum of squared residuals produced by the inclusion of more parameters in the model is statistically significant, the F statistic is calculated:

$$F_{\mathrm{calc}} = \frac{(\mathrm{SC_s} - \mathrm{SC_c})/(\nu_{\mathrm{s}} - \nu_{\mathrm{c}})}{\mathrm{SC_c}/\nu_{\mathrm{c}}} \tag{4.3.60}$$

where $\mathrm{SC_s}$ and $\mathrm{SC_c}$ correspond to the sum of squares of the simple and complex model respectively, and υ_{s} and υ_{c} are the degrees of freedom of each model (number of data points minus number of parameters).

The calculated value of F is compared with the tabulated F for the desired probability level (in general 0.05) and the degrees of freedom of the numerator and denominator. If the calculated F is lower than the tabulated F, then the decrease of the sum of squares is not significant so the simplest model should be chosen. When the calculated F value is higher than the tabulated one, then the improvement in the sum of squares is statistically significant, i.e. the complex model is better.

Appendix 4.3.3: Software tools, companies and institutions developing biosimulation packages

Adapted from Rowland (Rowland *et al.*, 2004); other URL link including modelling software: www.boomer.org/pkin/soft.html

General tools for scientific computing

- Berkeley Madonna, University of California at Berkeley: www.berkeleymadonna.com/
- MATLAB-Simulink, The MathWorks, Inc: www.mathworks.com
- MLAB: Civilized Software, Inc: www.civilized.com/
- GNU Octave, University of Wisconsin: www.octave.org/

Biomathematical and pharmacokinetics modelling software

- ADAPT II, Biomedical Simulations Resource, USC: bmsr.usc.edu/
- ModelMaker, ModelKinetix: www.modelkinetix.com
- NONMEM, Univ. of California, San Francisco and Globomax Service Group: www.globomaxservice.com
- Stella High Performance Systems Inc: www.hps-inc.com/
- WinNonlin, WinnonMix, Trial Simulator, Pharsight Corp: www.pharsight.com/products/prod_home.php
- IntellipharmPK, IntellipharmCR, IntellipharmC: www.intellipharm.com/

Toxicokinetic software

- ACSL Toxicology Toolkit, AEgis Technologies Group Inc: www.aegistg.com/

Physiologically based custom-designed software

- Simulations Plus Inc: www.simulations-plus.com
- SimCyp: www.simcyp.com

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