CHAPTER 9

BIOEQUIVALENCE AND BIOPHARMACEUTICAL CLASSIFICATION SYSTEM

"By far the best proof is experience."

—Francis Bacon

9.1 BIOEQUIVALENCE

The equivalence of bioavailabilities from two different formulations of the same drug is referred to as *bioequivalence* (BE). Even when two formulations contain the same drug, the C_p–time profiles after dosing these formulations could be different. Therefore, it is critically important to confirm the BE of C_p–time profiles when the formulation is changed during drug development (including generic drug development).

A standard BE study employs a crossover design in 12–24 healthy volunteers. A crossover design is employed to avoid the interindividual variations in drug disposition processes. The bioavailability of a drug product (both rate and extent of oral absorption) should be identical between the two formulations (Fig. 9.1). Definition of *in vivo* BE is

$$80\% < C_{max} < 125\% \text{ (with 90\% confidence interval)}$$
$$80\% < AUC < 125\% \text{ (with 90\% confidence interval).}$$

Biopharmaceutics Modeling and Simulations: Theory, Practice, Methods, and Applications, First Edition. Kiyohiko Sugano.

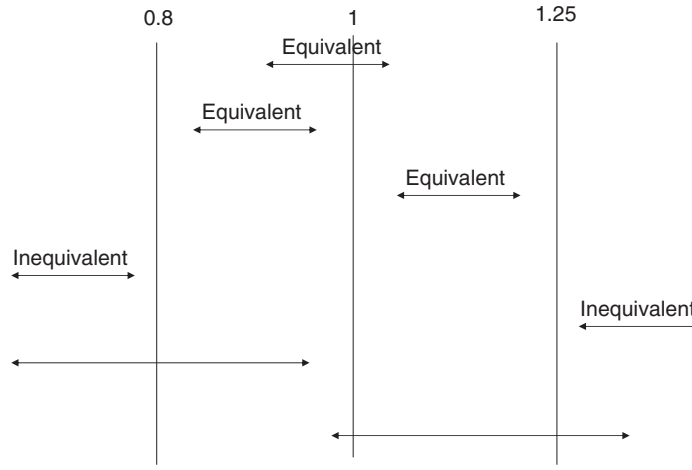

Figure 9.1 90% confidence interval and bioequivalence.

However, it is practically impossible to confirm BE by a clinical study every time the formulation is changed. The clinical BE study is expensive (ca. $100,000–250,000) and time consuming, which could in return be reflected in the drug price. In addition, it is ethically preferable to reduce the number of clinical studies with healthy volunteers. Therefore, the appropriate use of an *in vitro* dissolution test to waive (be exempted from) a clinical BE study would be of great benefit for both patients and industries [1]. However, it is well known that an *in vitro* dissolution test is not versatile for all drug products. Therefore, a guidance on when and for what case an *in vitro* study can be used to ensure BE is important.

The Biopharmaceutical classification system (BCS) was used as such guidance, first by the US FDA, and later by EMEA (EMA) and WHO. BCS has been widely known as a concept to classify a drug molecule on the basis of equilibrium solubility and effective permeability. Since the original publication in 1995 by Amidon and coworkers, this concept has been applied to various situations all through drug discovery and development [2, 3]. At present, BCS is used as a common language in the pharmaceutical industries, regulatory agencies and academia. At the same time, the discussion about BCS is often confused, as it is interpreted from different points of view.

BCS was derived from the same theories used in biopharmaceutical modeling. Therefore, in this section, as an important application of biopharmaceutical modeling, the BCS concept and its position in drug discovery and development are discussed. The concept of BCS is currently used for both regulatory and exploratory drug discovery perspectives, named regulatory BCS and exploratory BCS, respectively. The regulatory BCS is used in regulatory submission for biowaiver.[1] The formal BCS criteria are strictly defined in the regulatory

[1]After the candidate selection, especially after FIH, people tend to use the word "BCS" in this context.

guideline of each administrative region. Extensive and rigorous experimental investigations are required to define the class of a drug in the regulatory BCS. On the other hand, the exploratory BCS is more conceptually used in drug discovery and roughly defined on the basis of a simple *in vitro* experiment or even an *in silico* prediction.[2] In the following sections, we start with the original articles from the Amidon's group and then move forward to recent progresses.

9.2 THE HISTORY OF BCS

Probably, the origin of the BCS concept may be traced back to the 1960s or earlier. It had been well recognized that both solubility and permeability of a drug affect its oral absorption. Since then, there have been various theoretical and experimental investigations to understand the quantitative relationship between solubility, dissolution rate, permeability, and oral absorption of a drug. However, it remained unclear as to what parameters are essential to characterize the oral absorption of a drug. Especially, the dual role of solubility, that is, to determine both the maximum dissolved drug concentration and the dissolution rate, might have made it difficult to comprehend the relationships between each parameter.

In 1993, a pivotal paper was published by Amidon and coworkers [4]. They theoretically proved that three dimensionless parameters, that is, the dose number (Do), the dissolution number (Dn), and the absorption number (An), are sufficient to determine the oral absorption of a drug (for the cases without precipitation). They applied the plug flow model and rearranged the dissolution and permeability equations by introducing the above three dimensionless parameters. The following pair of differential equations was derived (Eqs. 9.1 and 9.2).[3]

$$\frac{\mathrm{d}r^*}{\mathrm{d}z^*} = -\frac{\mathrm{Dn}}{3}(1 - C^*)\frac{C^*}{r^*} \tag{9.1}$$

$$\frac{\mathrm{d}C^*}{\mathrm{d}z^*} = \mathrm{Do} \cdot \mathrm{Dn} \cdot r^*(1 - C^*) - 2\mathrm{An}C^* \tag{9.2}$$

where

$$z^* = \frac{z}{L_{\mathrm{GI}}}, C* = \frac{C_{\mathrm{dissolv}}}{S_{\mathrm{dissolv}}}, r* = \frac{r_{\mathrm{p}}(t)}{r_{\mathrm{p,ini}}} \tag{9.3}$$

$$\mathrm{Do} = \frac{\mathrm{Dose}}{S_{\mathrm{dissolv}} \cdot V_{\mathrm{GI}}} \tag{9.4}$$

$$\mathrm{Dn} = k_{\mathrm{diss}} T_{\mathrm{si}} = \frac{3\mathrm{D}_{\mathrm{eff}} S_{\mathrm{dissolv}}}{r_{\mathrm{p,ini}}^2 \rho} T_{\mathrm{si}} \tag{9.5}$$

[2]Before the candidate selection in drug discovery, people tend to use the word "BCS" in this context.
[3]As a courtesy for the original paper, An is used here. In the other parts in this book, permeation number (Pn) is used to simplify the equation.

$$\mathrm{An} = \frac{1}{2} k_{\mathrm{perm}} T_{\mathrm{si}} \left(= \frac{1}{2} \mathrm{Pn} \right) \tag{9.6}$$

where z^*, C^*, and r^* are the dimensionless variants representing the position of the API particle in the GI tract, C_{dissolv}, and r_{p} at the position, respectively. By the plug flow model, the time after oral administration was converted to the position of API particles in the GI tract at the time (z^*) (cf. Eq. 5.2).

Even though Equations 9.1 and 9.2 are still sequential differential equations, all the coefficients are grouped into the three dimensionless parameters.[4] This means that the oral absorption of a drug is sufficiently described by these three dimensionless parameters.

$$\mathrm{Fa} = f(\mathrm{Do}, \mathrm{Dn}, \mathrm{Pn}) \tag{9.7}$$

This is the most important conclusion of the 1993 paper. This finding means that if the identity of Dn, Do, and Pn between the two formulations were shown, the BE of the two formulations could then be proved.[5] This congruent condition for BE is discussed in Section 9.3).

In the 1993 paper, the pair of the equations was then numerically solved[6] to investigate the shape of Equation 9.7 (Fig. 9.2). At present, we have an approximate analytical solution as [5, 6]

$$\begin{aligned} \mathrm{Fa}_{\mathrm{SS}} &= 1 - \exp\left(- \frac{1}{\dfrac{1}{k_{\mathrm{diss}}} + \dfrac{\mathrm{Do}}{k_{\mathrm{perm}}}} \cdot T_{\mathrm{si}} \right) \\ &= 1 - \exp\left(- \frac{1}{\dfrac{1}{\mathrm{Dn}} + \dfrac{\mathrm{Do}}{\mathrm{Pn}}} \right) \text{ If } \mathrm{Do} < 1,\ \mathrm{Do} = 1 \end{aligned} \tag{9.8}$$

In 1995, on the basis of the above-mentioned theoretical analysis,[7] the BCS classification was proposed by Amidon and coworkers [7]. In the original BCS paper, solubility, rather than the dose number, was first used for classification. Furthermore, the low solubility/high permeability class (today, this corresponds to BCS II) was described as "the cases where An is high and dissolution number

[4]This is in a similar situation of the Reynolds number (Re) in the Navier–Stokes equation. The parameters of the flow system, for example, main flow velocity (U), fluid viscosity (μ), fluid density (ρ), and representative length (L), are lumped into Re as $\mathrm{Re} = UL\rho/\mu$.

[5]This is something as proving the congruence of triangle by Angel-Side-Angle theorem.

[6]This is one of the earliest applications of computational biopharmaceutical modeling.

[7]The title of the first paper that underwrites the BCS is "Theoretical bases for BCS," however, not "BCS as theoretical bases of oral absorption." Sometimes BCS is referred to as a theory. However, this is misleading because BCS is the consequence of a theory but not the theory itself. The theoretical bases of BCS is Equations 9.1–9.6.

(Dn) is low." Currently, the dose number (Do) is used for low/high solubility definition. The quadrants of the BCS panel (Do-Pn[8] panel) were assigned a BCS class I–IV as (Fig. 9.3)

$$\text{BCS I} : \text{Do} < 1,\ \text{Pn} > 3$$

$$\text{BCS II} : \text{Do} > 1,\ \text{Pn} > 3$$

$$\text{BCS III} : \text{Do} < 1,\ \text{Pn} < 3$$

$$\text{BCS IV} : \text{Do} > 1,\ \text{Pn} < 3.$$

In 1999, another classification scheme was introduced by Yu et al. [8]. In the BCS classification, the dissolution number (Dn) is not taken into account. In Yu's classification, the dissolution-rate-limited (DRL) and solubility-permeability limited cases are separated. Currently, we define them as

$$\text{Dissolution-rate limited} : \text{Dn} < \text{Pn}/\text{Do}$$

$$\text{Permeability limited} : \ \text{Pn} < \text{Dn}, \text{Do} < 1$$

$$\text{Solubility–permeability limited} : \ \text{Pn}/\text{Do} < \text{Dn}, \text{Do} > 1.$$

A drug being classified as BCS II does not mean that its oral absorption is categorized as "DRL." Actually, the majority of BCS class II drugs are "solubility–permeability limited" (Section 9.5.2). Furthermore, even in the case of BCS class I and III drugs (i.e., Do $<$ 1, high solubility), the oral absorption could be "DRL." For example, for a drug with a dose strength of 1 mg, solubility of 0.05 mg/ml, particle diameter of 200 μm, and $P_{\text{eff}} = 3 \times 10^{-4}$ cm/s, the oral absorption would be DRL even though Do is less than 1 (Do $= 0.2$, Dn $= 0.4$, Pn $= 8.6$).

9.3 REGULATORY BIOWAIVER SCHEME AND BCS

The regulatory BCS is used in the biowaiver scheme (BWS) for a BE study. Currently, the following two-step strategy is employed as the BWS in several regulatory bodies.

BWS step 1: BCS classification

BWS step 2: *In Vitro* dissolution test.

[8]The original criteria of permeability is defined by Fa% (high permeability is Fa% $>$ 90%). If there is no solubility/dissolution-rate limitation and absorption occurs homogeneously in the small intestine, there is one to one relationship between Fa% and Pn as Fa $= 1 - \exp(-\text{Pn})$. Therefore, in this book, Pn is used instead of Fa%. Fa% is often not available, especially for the solubility-limited and DRL cases. Therefore, Pn is more realistic as a permeability parameter. Caco-2 permeability has been used as the surrogate of Fa% in regulatory submission. As you see in this section, the use of Pn makes the discussion much more straightforward and comprehensive. Fa% is a time-dependent parameter (T_{si} dependent) but not a thermodynamic (equilibrium) parameter.

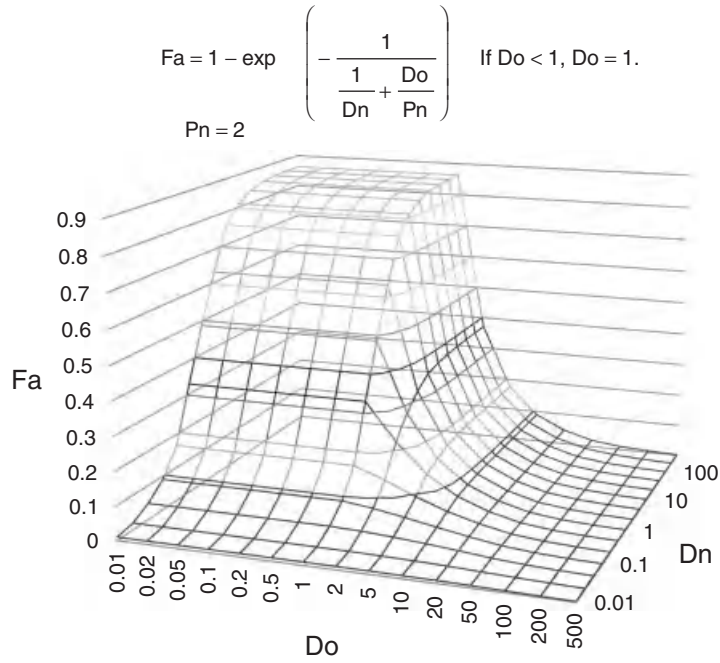

Figure 9.2 Shape of Fa function calculated by Fa_{SS} equation.

The first step is the classification of an active pharmaceutical ingredient (API) by dose/solubility ratio and permeability. BCS I drugs are granted permission to proceed to the second step (FDA guideline).[9] The second step is a dissolution test. An official dissolution test apparatus is used to confirm the identity of the dissolution process (e.g., USP paddle, 50 rpm, 900 ml, 85% dissolution in 30 min).

However, the scientific rationale behind applying this two-step process is not self-evident. Why is it acceptable to use an *in vitro* dissolution test for BCS I drugs but not for BCS II–IV drugs? In the following sections, this point is discussed in detail.

9.3.1 Elucidation of BCS Criteria in Regulatory Biowaiver Scheme

In the original BCS paper published in 1995, it was written that "(this analysis) clarifies the regime of the drug absorption process and offers a basis for determining when and under which condition *in vitro*–in vivo correlation are to be expected." [7]. Later in 2002, Yu of FDA wrote [9]: "When combined with the *in vitro* dissolution characteristics of the drug product, the BCS takes into account three major factors: solubility, intestinal permeability, and dissolution

[9]A clinical BE study is required for BCS II–IV drugs.

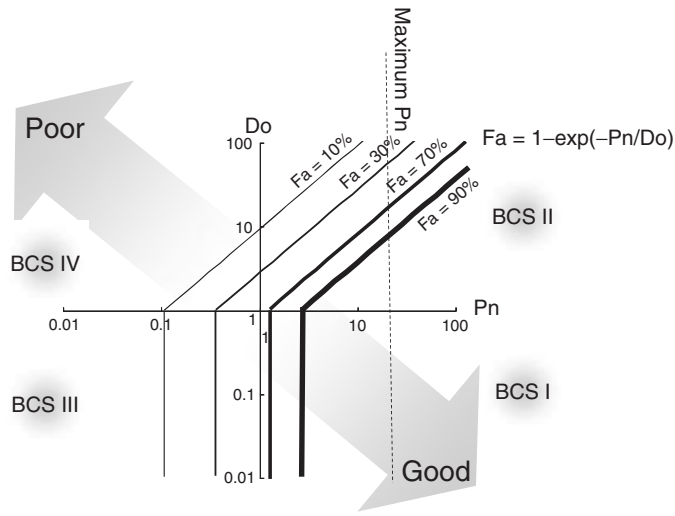

Figure 9.3 Fa% contour line in the BCS plane (with no dissolution-rate limitation).

rate, all of which govern the rate and extent of oral drug absorption from IR solid oral-dosage forms." In this section, based on the Yu's assertion, a further elucidation of the BCS criteria is attempted.[10]

9.3.1.1 Congruent Condition of Bioequivalence. Let us assume that a compendium *in vitro* dissolution test can be used to prove the equivalence of the *in vivo* dissolution number (Dn) as in the BWS step 2.[11] If Do and Pn were proved to be equivalent between the test and reference products, the two drug products can be proved to be bioequivalent by the congruent condition of BE (Fig. 9.4). The next question is to define the conditions by which a formulation change would not affect Do and Pn.

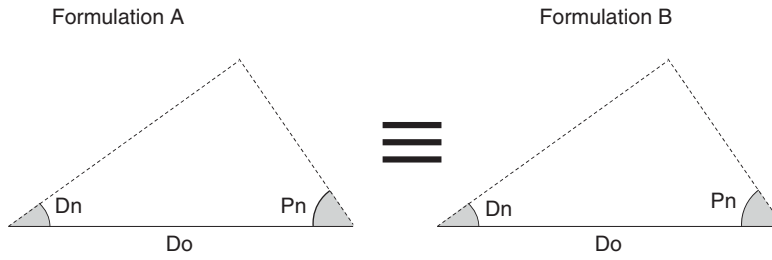

Figure 9.4 The congruent condition of bioequivalence.

[10]The following discussions are personal opinions.
[11]But this assumption is questionable.

9.3.1.2 Equivalence of Dose Number (Do). When the solubility of an API is sufficient to completely dissolve the drug in the stomach and the small intestine, the dose number becomes less than 1. In the approximate Fa% equation (Eq. 9.8), if Do is less than 1, regardless of the Do value, it is reset to 1. This means that when Do < 1, it does not affect the Fa%. Therefore, the equivalence of Do in Fa% equation can be proved if Do < 1 for both the drug products. Usually, excipients do not reduce the solubility of a drug. Therefore, it is sufficient to show the Do value of an API to be less than 1. The Do value for regulatory BCS is calculated based on the equilibrium solubility of a drug at physiological gastrointestinal pHs (pH 1.2–6.8 or 7.4) at 37° C, the highest dose strength, and a fluid volume of 250 ml.[12]

9.3.1.3 Equivalence of Permeation Number (Pn). Similarly, we can discuss the conditions in which Pn is not affected by the formulation. As discussed in Chapter 4, the rate-limiting process of membrane permeation can be categorized into the unstirred water layer (UWL) and epithelial membrane permeations. The UWL diffusion is a simple diffusion process through the aqueous layer, and it is highly unlikely that any excipient will change the diffusion rate through this layer. On the other hand, it is possible that some excipients affect the epithelial membrane permeability by changing the lipid membrane fluidity or by affecting the carrier-mediated transport of a drug. The effective permeability (P_{eff}) becomes UWL limited when $P_{\text{eff}} > 2 \times 10^{-4}$ cm/s. Therefore, Pn > 5.5 would be sufficient to show the equivalence of Pn between the two drug products.[13] Currently, metoprolol ($P_{\text{eff}} = 1.3 \times 10^{-4}$ cm/s, Pn $= 3.7$) is used as a marker for high/low permeability. For methanol, it was suggested that about 50% of the permeation resistance was from the UWL [10]. Originally, the permeability criteria was defined based on Fa% in humans. In FDA regulatory guidance, Fa% $> 90\%$ is used as the permeability criteria, which corresponds to Pn $= 2.3$.

In the biopharmaceutical drug disposition classification system (BDDCS) [11, 12], when the total amount of oxidative and conjugate metabolites is $> 90\%$, the drug is categorized as high permeability (cf. drug metabolism usually occurs after absorption into the body).

In addition, Pn also affects the criteria setting of Dn equivalence in two different meanings: (i) the setoff effect of dissolution rate difference between two formulations and (ii) the maintenance of sink condition *in vivo*. These points are discussed in the next section.

9.3.1.4 Equivalence of Dissolution Number (Dn). Once the equivalences of Do and Pn are proved in BWS step 1, we can proceed to BWS step 2, the dissolution test. If the dissolution numbers (Dn) of the two drug products are

[12]The equilibrium solubility at a pH is basically identical, regardless of the starting material being a salt or free from. On the other hand, the intrinsic dissolution rate depends on the API being free or salt. Therefore, the intrinsic dissolution rate cannot be used for BCS.

[13]Extension of permeability criteria to low–moderate permeability (BCS class III) is possible, if we can prove that the excipients used in the formulation do not alter the permeability of a drug.

proved equivalent in BWS step 2,[14] as the equivalences of Do and Pn have already been proved in BWS step 1, we can then prove the *in vivo* BE of the two formulations in accordance with the congruent condition for BE. However, unlike Do and Pn, a mechanistic consideration cannot be used to show the equivalence of Dn. Therefore, the equivalence of Dn has to be proved by an *in vitro* dissolution test. For this purpose, the *in vitro* dissolution test should reflect the *in vivo* conditions. The dissolution process of a drug product *in vivo* is affected by various factors such as pH, buffer species, surfactant, shear force, and destructive force. However, a simple compendium dissolution test cannot capture all the factors of the *in vivo* dissolution. Therefore, we should carefully design a dissolution test. Usually, the discrimination power of a dissolution test is as follows:

- Weak agitation > strong agitation
- Less solubilizer > more solubilizer
- Sink condition > nonsink condition.

If the Dns of the two formulations are diagnosed as equivalent under the conditions that are more discriminative than the *in vivo* situation, we can expect that the two formulations will show the same dissolution profiles *in vivo*. However, because of uncertainty in the similarity of *in vitro* and *in vivo* conditions, a safer criterion is used in regulatory guidelines.

Rapid dissolution (>85% dissolution in 30 min) is employed in the FDA, EMEA, and WHO guidelines for BCS I. In addition, very rapid dissolution (>85% dissolution in 15 min) is also used in the WHO guideline for BCS III. The rationale of the rapid and very rapid dissolution criteria is that if the mean dissolution time (MDT) of the two formulations is much smaller than the intestinal transit time (ca. 210 min), the effect of the difference of the dissolution rates on Fa% would be minimum (hence Dn can be considered to be equivalent).

The relationship between the dissolution rate, permeability, and BE has been investigated using biopharmaceutical modeling [13–15]. Figure 9.5 shows the effect of mean dissolution time ($\mathrm{MDT} = 1/k_{\mathrm{diss}}$) and mean permeation time ($\mathrm{MPT} = 1/k_{\mathrm{perm}}$) on the maximum difference of Fa% (i.e., instant dissolution vs 85% dissolution at 15 or 30 min) (Do < 1). When permeability is higher (MPT is smaller), the difference in Fa% becomes smaller. When the very rapid dissolution criterion is used (85% dissolution at 15 min), the difference in Fa% becomes less than 20%, regardless of the permeability of the drug. When the rapid dissolution criterion is used (85% dissolution at 30 min), the permeability number of the drug needs to be larger than 2.3 ($P_{\mathrm{eff,human}} > 0.8 \times 10^{-4}$ cm/s) for the difference of Fa% to be less than 20%.

Currently (as of September, 2011), in the FDA, EMEA, and WHO guidelines, a sink condition is required for the dissolution test. A sink condition is defined as the dissolved drug concentration being less than 30% of the equilibrium solubility in the fluid. The Do < 1 condition in BWS step 1 automatically guarantees a sink

[14]Here, we include the disintegration process in Dn.

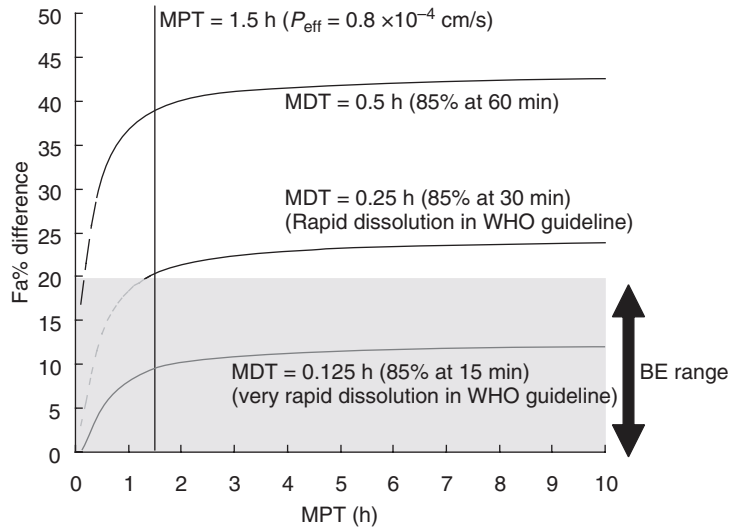

Figure 9.5 The relationship between Fa% difference, MPT, and MDT.

condition in the 900-ml dissolution test (cf. Do is calculated based on 250 ml volume). However, Do < 1 does not guarantee a sink condition *in vivo*, especially when the permeability of a drug is low (therefore, the criterion should be Do < 0.3 for BCS III). The high permeability (Pn > 3) works to maintain a sink condition *in vivo*.

9.3.2 Possible Extension of the Biowaiver Scheme

Historically, the BCS criteria was set conservative and the biowaiver was granted only for BCS class I drugs. At the same time, the expansion of applicable BCS category has been investigated.

9.3.2.1 Dose Number Criteria. Currently, Do is calculated on the basis of the minimum equilibrium solubility between pH 1.2 and 7.4 (or 6.8). However, as the main absorption site is the small intestine, Do < 1 at neutral pH in the small intestine is expected to be sufficient to prove the BE. Many NSAIDs that have Do > 1 in the stomach and Do < 1 in the intestine show a complete oral absorption after oral administration [16–18].

The use of a biorelevant media such as FaSSIF would be more suitable to judge the dose number for drugs with low solubility [19].

Ideally, both *in vitro* and *in vivo* dissolutions should be under a sink condition. Therefore, Do < 0.3 would be safer than Do < 1. Considering that absorption of the dissolved drug into the body can enhance the *in vivo* sink condition, Sn < 0.3 would be a more suitable criteria to judge the *in vivo* sink condition (cf. $Sn = 1/(1 + Pn/(Dn \times Do))$, Eq. 5.32).

In addition, a formulation change does not always affect the solubility of a drug in the intestinal fluid. Many excipients are actually inert to the solubility of a drug. Therefore, if the formulation change is limited to these inert excipients, it is unlikely that a formulation change will affect Do. A nonsink dissolution test using a small fluid volume (100–250 ml) might be able to be assess the equivalence of Do for Do >1 cases.

Recently, it was suggested that the supersaturation of a drug should be taken into account in BCS [20, 21]. This could potentially increase the number of applicable drugs for biowaiver. However, considering that the science of nucleation is not well understood and the *in vitro* dissolution test is not suitable to assess the nucleation, it would not be appropriate to expand the BCS criteria based on the critical supersaturation concentration.

9.3.2.2 Permeability Criteria. There are several points to be considered when expanding the permeability criteria [22, 23]. They are as follows:

1. *The Effect of Excipients on the Epithelial Cellular Membrane Permeability*. Most excipients are actually inert to the permeability for passive transport case. However, the effect of excipients on carrier-mediated transports is not well known. In addition, the effect of excipients on the GI physiology (such as GI mobility) is also not well known and needs further investigations [24].
2. *The Relationship with the Dissolution-Rate Criterion*. As discussed above, theoretically, the permeability criterion affects the dissolution test criterion.
3. *Maintenance of Sink Condition by Permeability*. For drugs with low permeability, its absorption is not effective to maintain a sink condition in the *in vivo* small intestine. Therefore, Do < 0.3 would be a safer criterion to guarantee a sink condition *in vivo* for drugs with low permeability.

9.3.3 Another Interpretation of the Theory

In the previous section, the mainstream opinions about the BWS, the BCS BWS, are discussed. In this section, this topic is discussed with a different logical plot.

9.3.3.1 Another Assumption about Dissolution Test. The discussion about the BCS BWS is based on an assumption that the equivalence of the dissolution number (Dn) can be assessed by a compendium dissolution test. However, most of the practical formulation scientists think that it may not be a valid assumption. Considering the simple paddle apparatus and artificial dissolution test media being in contrast to the complex *in vivo* GI physiology, it is obvious that the *in vitro* paddle method cannot represent all factors of the *in vivo* dissolution. The 50-rpm paddle method is most often used in the dissolution test. However, it was reported that the agitation strength in humans corresponds to 10–30 rpm (Section 6.2.3). At present, extensive investigations are underway to develop more biorelevant dissolution tests [25]. On the other hand, as long as standard excipients, which are usually inert for equilibrium solubility and

permeability of a drug, are used, the dose number (Do) and permeation number (Pn) should not be affected by the difference of the two formulations. Therefore, the proof of the equivalence for the dissolution number (Dn) would be the most uncertain among the three parameters. Once this assumption is agreed upon, the next question is what kinds of drugs are less sensitive to the uncertainty of the Dn equivalence.

9.3.3.2 Assessment of Suitability of Dissolution Test Based on Rate-Limiting Process. As discussed in the previous sections, the oral absorption of drug products can be categorized as DRL, permeability limited (PL), and solubility–permeability limited (SL). Solubility–permeability limited can be further categorized by the rate-limiting process in permeability, that is, solubility UWL limited (SL-U) and solubility epithelial membrane permeability limited (SL-E).

Even though it is counterintuitive, theoretically, the oral absorption of an SL drug should be less sensitive to the uncertainty of the Dn equivalence, as Pn/Do dominates the oral absorption of the drug. This class of compound corresponds to BCS II or IV. When we compare UWL and epithelial-membrane-limited cases in this class (i.e., SL-U and SL-E, respectively), the former case is possibly sensitive to the particle size of the API because of the particle drifting effect. Therefore, SL-E should be most insensitive to a change in formulation. In addition, the solubility of drugs in this class is less sensitive to the surfactant, as the main reason for low solubility would be high crystallinity but not lipophilicity (Section 2.3.7). SL-E corresponds to BCS class IV. Therefore, BCS class IV would be the most probable case where *in vitro* equivalences of the three parameters can be translated to *in vivo* equivalence. This is the opposite of what is suggested by the BCS BWS.

The DRL case is most sensitive to the differences between *in vitro* and *in vivo* dissolution conditions. Therefore, if an *in vitro* dissolution test is not a good representation of an *in vivo* situation, an appropriate IVIVC should not be observed. In a permeability-limited case (Pn < Dn), the dissolution rate can affect the C_{max}, as the flux $= C_{dissolv} \times P_{eff}$ and $C_{dissolv}$ in the early stages of oral absorption largely depends on the dissolution rate. If the *in vitro* dissolution rate of a drug is threefold faster than the *in vivo* dissolution rate, 85% dissolution in 30 min *in vitro* could be 30% dissolution *in vivo*. Figure 9.6 shows such a case.

9.3.4 Validation of Biowaiver Scheme by Clinical BE Data

The most conservative criterion for Do and Pn, that is, "Do < 1 and Pn > 2.3," was originally adopted by the FDA. As discussed above, this BCS BWS is based on the theoretical consideration, and therefore, needs to be validated experimentally. What percentage of biowaivered drug products actually shows clinical BE in healthy volunteers (and even in patients)? This type of investigation is critical for the future improvements of the BCS BWS.

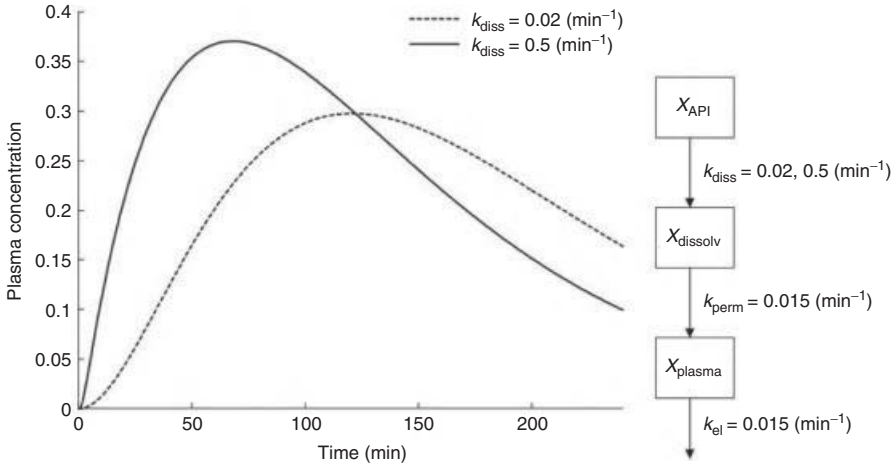

Figure 9.6 Effect of dissolution rate on C_{max} for a BCS III drug. Simulation results based on three sequential first-order kinetics (k_{diss}, k_{perm}, and k_{el}).

Recently, the result of a validation study of the BCS scheme was reported [26]. The results of 124 clinical BE studies were statistically analyzed based on the BCS classes. As expected from both the BCS and rate-limiting process discussions, BCS II showed the largest percentage of clinical inequivalence. However, BCS I showed ca. 15% false-positive results. All of them failed BE in C_{max} but not in AUC. This ratio is similar for BCS class III. Surprisingly, even though the number of samples is small, six out of seven BCS IV cases showed clinical BE.

9.3.5 Summary for Regulatory BCS Biowaiver Scheme

In summary, even though the final clause of the BCS BWS looks different for each regulatory body, the basic concept for biowaiver is the same, that is, "Fa% is determined by Dn, Do and Pn. If a formulation change has no effect on all Dn, Do and Pn, the two products will be bioequivalent" [9]. In the BCS-based BWS, the BCS classification is used to diagnose unlikeliness that a formulation change would affect Do and Pn. The compendium dissolution test is used to diagnose equivalence in Dn. Even though FDA, EMA, and WHO have the same structure of the BWS, that is, BCS + dissolution test, each regulatory agency has different criteria. Furthermore, another logical scenario can be derived from the same theory of oral absorption.

The BCS BWS should be further experimentally validated in the future. Meanwhile, for the safety of patients, it might be another good judgment to request a clinical BE study for the first launch of a generic drug product or after a significant change in formulation and manufacture process.

Even though the scientific basis is the same, the regulatory scheme in each administrable region can be different depending on the tolerance of the nation for

the risk and cost, which depends on the culture, history, ethnic difference, medical care and insurance schemes, etc. The regulatory scheme should be determined by the sovereignty of the nation. In addition, the patients have the right to know whether the drug product is approved based on the BWS or a clinical BE study [27],[15] and the freedom of choice.

9.4 EXPLORATORY BCS

The BCS concept has been widely used in drug discovery. In the lead optimization process, the BCS plane can be used to navigate the SAR. Combination of PAMPA and high throughput solubility screening was found to be able to give appropriate BCS classification [28].

The BCS concept is also applied to judge the developability of a drug [29]. The BCS plane can be used to diagnose whether the standard particle size reduction would result in a good Fa% or a special formulation will be required for the development. Particle size reduction down to 10 μm is usually achieved using a standard milling technology and will not be a development issue. In Equation 9.8, if the Do/Pn term is greater than 1, even when particle size is reduced ($Dn \gg 1$), the Fa% would not exceed 60% (as $1/Dn + Do/Pn$ cannot exceed 1). This criterion is determined by the ratio of Do and Pn. In other words, high permeability can compensate low solubility to give adequate Fa%. This concept is basically the same as that proposed by Lipinski based on the MAD calculation, which suggests that low/high solubility criteria for drug development change with the permeability and the dose strength of a drug [30].

9.5 *IN VITRO–IN VIVO* CORRELATION

IVIVC can be used as a biowaiver approach for extended-release formulations, as the release process becomes the rate-limiting step, but not the solubility and permeation.

9.5.1 Levels of IVIVC

The levels of IVIVC have been defined in the FDA guidelines as follows:

- Level A correlation is based on the relationship between *in vitro* and *in vivo* dissolved %. *In Vivo* dissolved % can be obtained by a deconvolution method such as the Wagner–Nelson, Loo–Riegelman, and model-independent numerical deconvolution methods (Section 5.5.4).
- Level B correlation is the relationship between the mean *in vitro* dissolution time ($MDT_{in\ vitro}$) of a product and the mean *in vivo* residence time (MRT) or the mean *in vivo* dissolution time ($MDT_{in\ vivo}$).

[15]In Japan, this information is available in the label or the interview form of generic products.

- Level C correlation is the relationship between one dissolution time point (e.g., $t_{50\,\%}$) and one mean pharmacokinetic parameter such as AUC, $T_{\max}$, or $C_{\max}$.

9.5.2 Judgment of Similarity Between Two Formulations (f2 Function)

The $f2$ function is most often used to quantify the similarity between the dissolution profiles of two formulations. The similarity factor ($f2$) can be calculated as

$$f2 = 50 \times \log_{10}\left(\left(1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right)^{-0.5} \times 100\right) \tag{9.9}$$

where n is the number of the sampling time points and R_t and T_t are the dissolved % values at time t for reference and test formations, respectively. The $f2$ becomes 100 when the dissolution profiles of two formulations are identical. When the dissolved fraction differs by 10% at each sample point, $f2 = 50$. When $50 < f2 < 100$, the two formulations are thought to have equivalent dissolution profiles.

9.5.3 Modeling the Relationship Between f2 and Bioequivalence

The relationship between the *in vitro* similarity ($f2$) and *in vivo* BE has been recently investigated using computational simulation [31]. In this section, a simpler approach is taken to correlate *in vitro* similarity and *in vivo* equivalence.

An IVIVC is expected most likely in the cases of DRL absorption. Therefore, by replacing k_a with k_{disso} in Equation 5.41, we obtain

$$C_p(t) = \frac{\text{Dose} \times \text{FgFh}}{V_d} \cdot \frac{k_{diss}}{k_{diss} - k_{el}}(\exp(-k_{el} \cdot t) - \exp(-k_{diss} \cdot t)) \cdots t \le T_{si} \tag{9.10}$$

$$C_p(t) = C_p(T_{si})\exp(-k_{el} \cdot (t - T_{si})) \cdots t > T_{si} \tag{9.11}$$

$$T_{\max} = \frac{\ln(k_{disso}/k_{el})}{k_{disso} - k_{el}} \tag{9.12}$$

The $f2$ was calculated after calculating the Dissolved % at the time points of 1/4, 1/2, 3/4, and 1 of the time of 85% dissolution.

$$\text{Dissolved\%} = 1 - \exp(-k_{diss} \cdot t) \tag{9.13}$$

These equations are used to calculate $C_{\max}$ of test and reference formulations with $k_{disso,test}$ and $k_{disso,reference}$, respectively. As shown in Figure 9.7, the range of $80\% < C_{max,test}/C_{max,reference} < 125\%$ overlaps the range of $f2 > 50$. Therefore, $f2 > 50$ is a good criterion to predict BE in clinical studies.

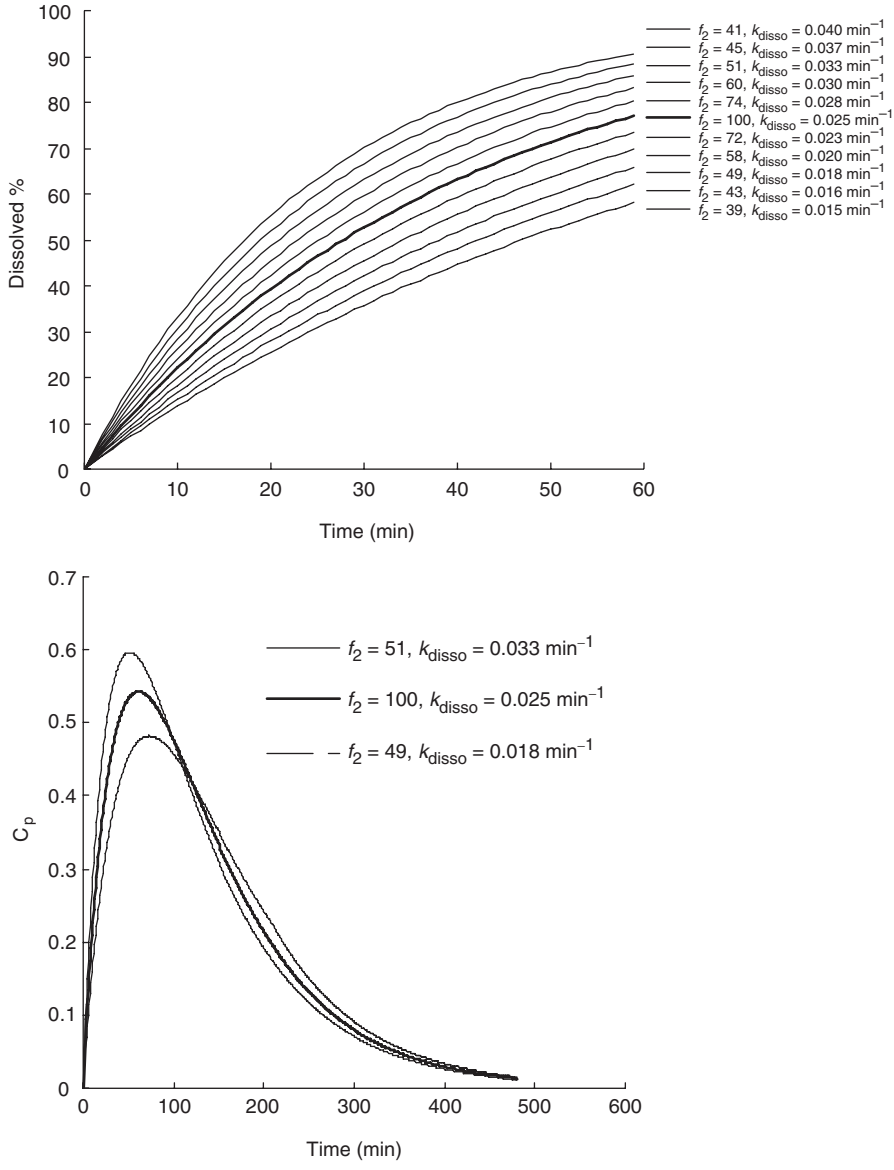

Figure 9.7 f_2 function and bioequivalence.

9.5.4 Point-to-Point IVIVC

The total amount dissolved until time t *in vitro* ($A_{in\ vitro}$) and the total amount absorbed until time t ($A_{in\ vivo}$) is often related using the following function.

$$A_{in\ vivo} = a1 + a2 \times A_{in\ vitro}(b1 + b2 \times t) \tag{9.14}$$

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CHAPTER 10

DOSE AND PARTICLE SIZE DEPENDENCY

"The whole of science is nothing more than a refinement of everyday thinking."
—Albert Einstein

Predictions of dose and particle size dependency are everyday requests in drug discovery and development. In preclinical toxicology and first-in-human (FIH) studies, a dose escalation study is usually performed. Compared to pharmacological studies, the maximum dose strength for these studies are significantly higher. For a preclinical toxicology study, 1000–2000 mg/kg dose is often required. For an FIH study, 1000 mg/dose or higher (>30 mg/kg) is often required. A preliminary toxicokinetic (TK) study is usually performed before the toxicological studies in each animal species. If the exposure is not sufficient, enabling formulation options are pursued (Chapter 11). In addition, simulation of particle size dependency is often requested in drug development, as the particle size of an API often becomes one of the key quality attributes.

10.1 DEFINITIONS AND CAUSES OF DOSE NONPROPORTIONALITY

In this book, the terms *dose linear* and *dose proportional* are used in the same meaning. When an increase in dose strength (e.g., two-, four-, and eightfold) results in a proportional increase in C_{max} and AUC (i.e., two-, four-, and

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eightfold), the pharmacokinetics (PK) is said to be "dose linear" and/or "dose proportional". A nonlinear PK can be caused by several reasons listed in the following.

Subproportional Exposure

- solubility-permeability-limited absorption;
- saturation of influx transporter in the intestine.

Supraproportional Exposure

- saturation of efflux transporters in the intestine;
- saturation of intestinal and liver first-pass metabolism;
- saturation of disposition CL (hepatic and renal clearances).

10.2 ESTIMATION OF THE DOSE AND PARTICLE SIZE EFFECTS

The dose and particle size dependency of Fa% can be estimated from the rate-limiting step of the oral drug absorption (Fig. 1.3) [1, 2].

10.2.1 Permeability-Limited Cases (PL)

In the permeability-limited absorption range (Do $<$ 1, Pn $<$ Dn), Fa% is usually independent of both dose and particle size, except for the cases in which a transporter is involved in the permeation process. The AUC and C_{max} linearly increase as the dose increases. However, as the dose exceeds a certain point, the dose number becomes larger than 1 and the absorption pattern could change from PL to solubility-limited (SL) absorption.

10.2.2 Dissolution-Rate-Limited (DRL) Cases

In dissolution-rate-limited (DRL) absorption (Dn $<$ Pn/Do), Fa% should be dose independent, but particle size dependent.[1] Particle size reduction would be effective in increasing the oral absorption of a drug. However, as the dose increases or particle size decreases, the regime of oral absorption could change from DRL to solubility-permeability-limited absorption.

The critical particle size discriminating DRL and SL can be calculated as follows. The criterion to discriminate DRL and SL, that is, 1/Dn $>$Do/Pn (for Do $>$1), can be rearranged to

$$\frac{1}{\mathrm{Dn}} = \frac{r_p^2 \rho}{3 \cdot D_{\mathrm{eff}} \cdot S_{\mathrm{dissolv}} \cdot T_{\mathrm{si}}} > \frac{\mathrm{Do}}{\mathrm{Pn}} = \frac{\mathrm{Dose}}{S_{\mathrm{dissolv}} \cdot V_{\mathrm{GI}}} \frac{R_{\mathrm{GI}}}{2\mathrm{DF} \cdot P_{\mathrm{eff}} \cdot T_{\mathrm{si}}} \tag{10.1}$$

[1]However, particle size dependency of AUC and C_{max} is not a sufficient condition to prove that the oral absorption is dissolution rate limited. The particle size could also affect the UWL permeability of a drug (particle drifting effect (PDE) (Section 4.7.2)).

By rearranging this equation, the critical radius to become DRL can be calculated as,

$$r_p > \sqrt{\frac{3D_{eff} \cdot \text{Dose} \cdot R_{GI}}{2 \cdot V_{GI} \cdot \text{DF} \cdot P_{eff} \cdot \rho}} \tag{10.2}$$

$S_{dissolv}$ is canceled out from the both sides of Equation 10.1, suggesting that the critical particle size does not depend on the solubility of a drug for Do >1 cases. This point can be interpreted as follows. When the solubility is low, the dissolution rate becomes slow, and at the same time, the ceiling of the dissolved drug concentration (=saturated solubility) becomes low. On the other hand, when the solubility is high, the dissolution rate becomes fast and the ceiling of the dissolved drug concentration becomes high. Therefore, the tendency of $C_{dissolv}$ reaching the saturated solubility (=becoming SL absorption) does not depend on the solubility of a drug (in other words, the tendency to deviate from the sink condition does not depend on $S_{dissolv}$).

Using a conventional milling process, the mean particle size can be reduced to 10 μm or less. The particle diameter of drugs with low solubility in the marketed formulation is often less than 10 μm. Therefore, according to Equation 10.2, even for relatively high P_{eff} cases such as 5×10^{-4} cm/s (before applying PDE), when the dose is greater than 20 mg (>0.3 mg/kg), the oral absorption becomes SL. As discussed in Section 8.5.2, the majority of BCS II drugs shows SL, but not DRL absorption. This is in good agreement with our real-life experiences in drug industries that a level A IVIVC (*in vitro* (dissolution)–*in vivo* correlation) is difficult to obtain for medium- to high-dose cases of drugs with low solubility (cf. DRL absorption is a prerequisite for a good IVIVC). During drug development, particle size reduction is usually used to remove dissolution rate limitation if incomplete oral absorption is anticipated.

It is often speculated that the particle size reduction would not be effective when the particle size is smaller than a critical value (i.e., oral absorption becomes solubility-permeability limited). However, this speculation is in contradiction with the experimental observations for the solubility-UWL-limited (SL-U) cases (Table 8.3). This point is discussed in Section 10.2.4.

10.2.3 Solubility–Epithelial Membrane Permeability Limited (SL-E) Cases

In this compound class, a steep dose dependency of Fa% is observed usually at a dose higher than that gives the dose number (Do) greater than 1. Figure 10.1 shows the dose dependency of Fa% for several solubility–epithelial membrane permeability limited (SL-E) drugs. When a Fa% information at a dose of Do < 1 is available, this data can be used to back-calculate the permeation number (Pn) (Section 7.9.1). This Pn is then used to calculate the Fa% at Do > 1 as $\text{Fa} = 1 - \exp(-\text{Pn}/\text{Do})$.

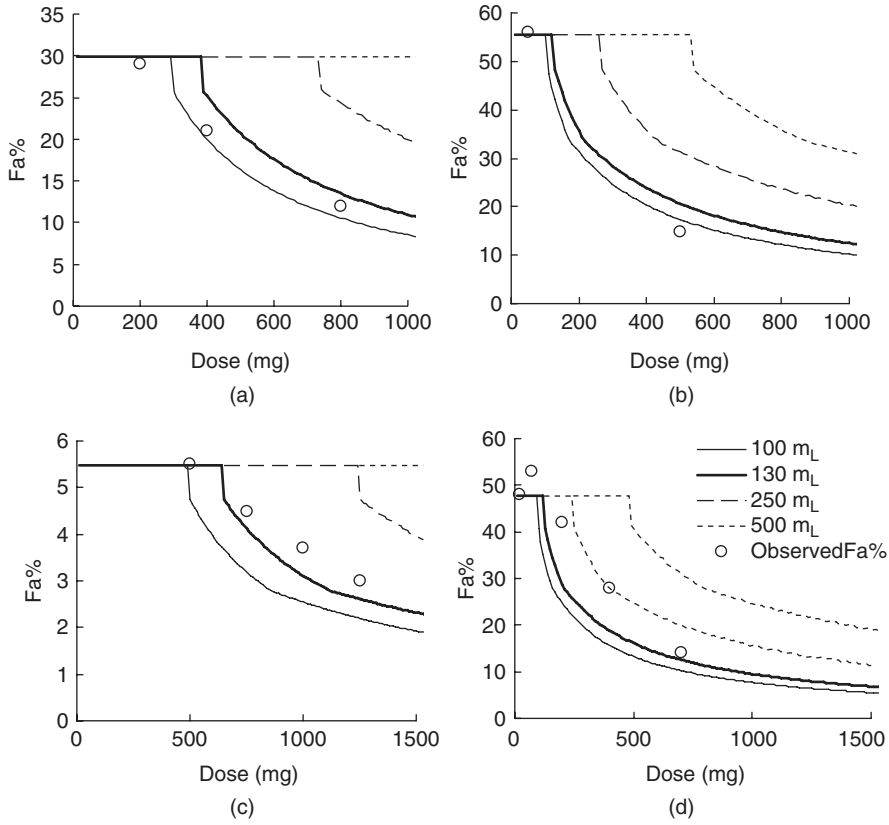

Figure 10.1 Dose dependency of Fa% for SL-E cases: (a) acyclovir, (b) chlorothiazide, (c) ganciclovir, and (d) lobucavir.

As shown in Figure 10.1, for the quantitative estimation of dose dependency for SL-E cases, it is critically important to use a correct V_{GI} value (100–250 ml) (Section 6.3.1.2). This V_{GI} corresponds to 5–15% of the full capacity of the small intestine (Fig. 4.3).

Example The inflection dose strength at which Fa% starts to decrease for acyclovir ($S_{dissolv} = 2.5$ mg/ml) can be calculated as

$$\text{Dose} > S_{dissolv} \times V_{GI} = 2.5 \times 130 = 325 \text{ mg}$$

In humans, Fa at 200-mg dose (Do $<$ 1) is 0.29. As $\text{Fa} = 1 - \exp(-\text{Pn}) \approx \text{Pn}$ for Do $<$ 1, Pn = 0.29. Fa for 800-mg dose (Do $>$ 1) is then estimated as

$$\text{Fa} = 1 - \exp(-\text{Pn/Do}) \approx \frac{\text{Pn}}{\text{Do}} = \frac{0.29}{800/325} = 0.12$$

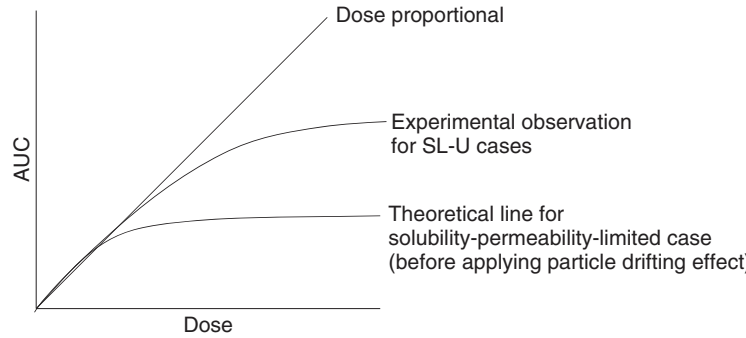

Figure 10.2 Discrepancy in the simulation of dose and size dependency for SL-U cases.

10.2.4 Solubility-UWL-Permeability-Limited Cases

In the solubility-permeability-limited cases, it was previously suggested that the absorbed amount (X_{abs}) should not increase with an increase in the dose strength since the concentration in the intestinal fluid becomes saturated (Fig. 10.2). As discussed above, this was proven for SL-E cases (Fig. 10.1). In addition, the previous theory also suggested that particle size reduction should not increase Fa% in the case of SL absorption.

However, for SL-U cases, these theoretical suggestions were inconsistent with experimental observations. The exposure of SL-U drugs is often (subproportionally) increased as their doses are increased without any change in the terminal half-life, for example, griseofulvin, celecoxib, efavirenz (Table 8.3). This is in clear contrast to the SL-E cases. In addition, particle size reduction was found to be effective in increasing Fa% for SL-U cases, for example, danazol and cilostazol (Table 8.3; Section 11.2, nano-API).[2]

These discrepancies may be due to overlooking the point that the drug particles can drift into the UWL (Section 4.7.2; Fig. 4.6). The PDE suggests that Pn could depend on the particle size and dose of a drug. Once this effect is taken into account, the dose and particle size dependency were reasonably simulated (Figs. 10.3–10.5; the other examples are found in Table 8.3). Using the same theoretical scheme, the effectiveness of particle nanomization to improve Fa% can be also elucidated (Section 11.2). A superficial rank order correlation (but not level A IVIVC) between the dissolution rate and *in vivo* oral absorption for SL-U cases can be a superficial correlation intermediated by the PDE.

10.3 EFFECT OF TRANSPORTERS

Dose-dependent absorption can be observed for a transporter substrate.[3] This point is discussed in detail in Chapter 14.

[2]This should not be confused with DRL.

[3]This does not mean that a transporter substrate always shows dose-dependent absorption. Apparent K_m could be higher or lower than the concentration range in the GI tract.

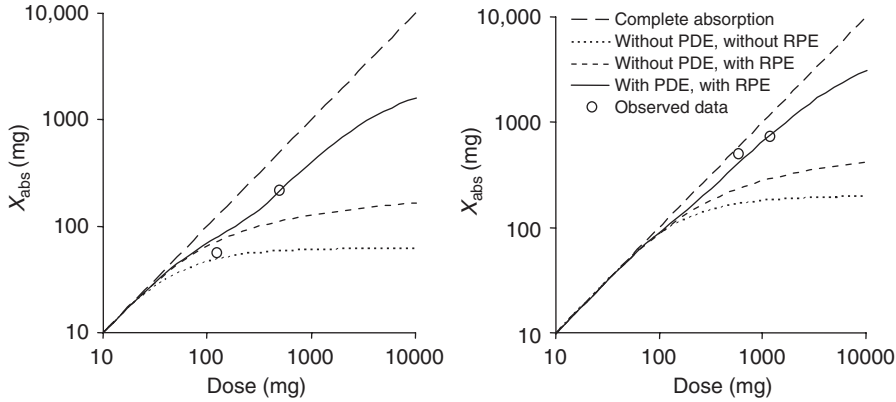

Figure 10.3 Dose dependency of Fa% for SL-U cases. RPE, remaining particle effect. (a) griseofulvin and (b) efavirenz. *Source*: Adapted from Reference [3] with permission.

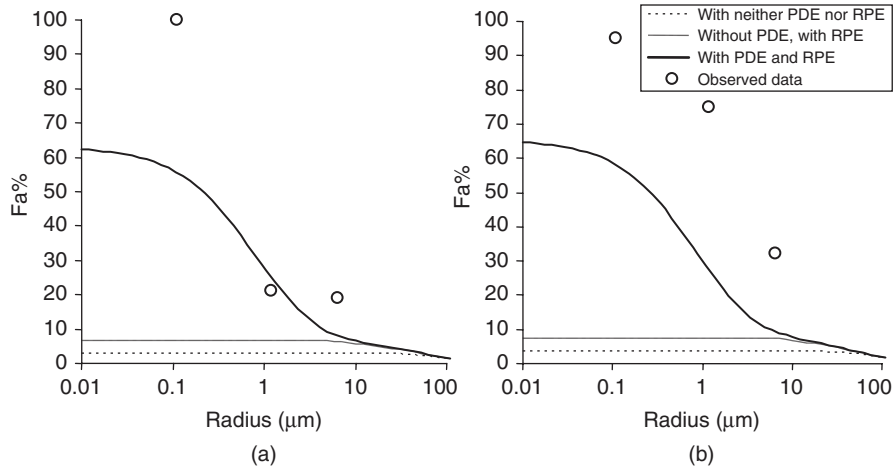

Figure 10.4 Particle size dependency of Fa% of cilostazol in dogs. The solid and dotted lines are the theoretical prediction with and without considering the particle drifting effect, respectively. (a) Fasted state and (b) fed state. *Source:* Adapted from Reference 3 with permission.

10.4 ANALYSIS OF *IN VIVO* DATA

In many cases, *in vivo* PK data at multiple dose strengths are available in drug discovery. If these data are carefully analyzed,[4] they give us a lot of information

[4]The formulation for PK and TK studies should be carefully prepared and well characterized (Section 7.10.1).

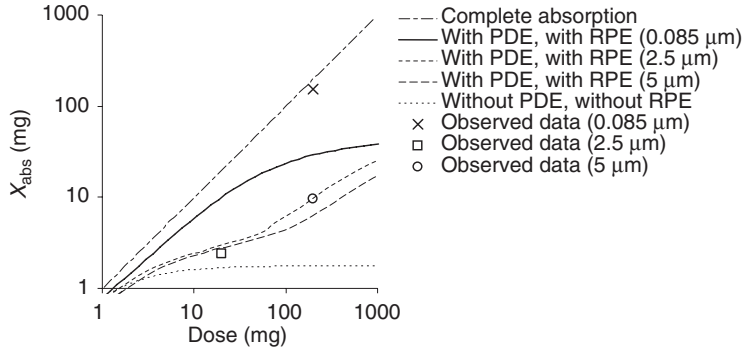

Figure 10.5 Dose and particle size dependency of Fa% of danazol in dogs. The solid and dotted lines are the theoretical prediction with and without considering the particle drifting effect, respectively. *Source:* Adapted from Reference [3] with permission.

about the performance of a drug. In addition, the information on log P_{oct}, solubility, permeability, and the metabolic and elimination pathways is of critical importance to investigate the dose–exposure relationship.

When AUC increases linearly (or supralinearly) with doses, it is highly unlikely that the oral absorption of the drug is limited by its solubility. In this case, further improvement of AUC by the current formulation technologies is not expected (unless otherwise DRL cases). However, dose subproportionality in the absorption process can be masked by dose suprapropportionality in a clearance process, resulting in superficial dose proportionality in AUC. This case can be ruled out by investigating elimination $t_{1/2}$. After normalizing AUC by $t_{1/2}$ (assuming Vd being consistent), the existence of dose subproportional absorption can be revealed (Section 5.5.3). However, even in this case, possibility of the saturation of intestinal fast-pass metabolism cannot be excluded. The information of the metabolic pathway can be helpful to evaluate the contribution of the intestinal first-pass metabolism (Section 4.10).

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