CHAPTER

# 16

# In Vitro/In Vivo Correlations: Fundamentals, Development Considerations, and Applications

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## 16.1 INTRODUCTION

For decades, developing in vitro tests and models to assess or predict the in vivo performance of pharmaceutical products has been sought after as a means of screening, optimizing, and monitoring dosage forms. With solid oral dosage forms, it most frequently starts with an attempt to link the results of an in vitro release test to in vivo pharmacokinetic studies. Through exploring the association or relationship between in vitro dissolution/release and in vivo absorption data, an in vitro/in vivo relationship (IVIVR) may be identified for a drug product. Such a relationship is often qualitative or semiquantitative in nature (eg, rank order). When a predictive relationship or model is established and validated between in vitro dissolution and in vivo absorption profiles, it is designated as in vitro/in vivo correlation (IVIVC).

IVIVC of oral solid products have received considerable attention from the industry, regulatory agencies, and academia over the past two decades, particularly since the publication of Food and Drug Administration (FDA) guidance of dissolution testing of immediate-release (IR) and IVIVC of extended-release (ER) dosage forms in 1997[1,2] and subsequent guidelines issued by European regulatory authorities (EMA).[3–5] As a result, there has been increased confidence, effort, and success using in vitro tests to evaluate or predict in vivo performance of solid drug products, especially ER dosage forms, based on IVIVC.[6–13] With an established IVIVC, the dissolution data can be used not only as a quality control tool but also for guiding and optimizing product development, setting meaningful specifications, and serving as a surrogate for a bioavailability study. This chapter will discuss basic principles and methodology utilized to establish and evaluating IVIVC models, as well as applications of IVIVC in developing solid dosage forms with a primary focus on ER products. Additional topics include the importance of understanding drug substance, dosage form, their in vitro and in vivo behaviors, and in vitro test methods to explore and develop IVIVC.

### 16.1.1 In vitro/in vivo correlation

IVIVC is defined by United States Pharmacopeia (USP) and the FDA respectively as follows[2,14]:

> *USP:* the establishment of a relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical characteristic of the same dosage form.
> *FDA:* a predictive mathematical model describing the relationship between an in vitro property (usually the extent or rate of drug release) and a relevant in vivo response (eg, plasma concentration or amount of drug absorbed).

Evaluation of IVIVCs by different levels was first proposed for oral dosage forms in the USP's information chapter <1088>[15] and was later adopted globally. Presently, IVIVC is categorized by the FDA into levels A, B, C, and Multiple C depending upon the type of data used to establish the relationship and ability of the correlation to predict the complete plasma profile of a dosage form.[2]

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*Level A*: A predictive mathematical model for the relationship between the entire in vitro release time course and the entire in vivo response time course, for example, the time course of plasma drug concentration or amount of drug absorbed.
*Level B:* A predictive mathematical model for the relationship between summary parameters that characterize the in vitro and in vivo time courses, for example, models that relate the mean in vitro dissolution time to the mean in vivo dissolution time, or to mean residence time (MRT) in vivo.
*Level C:* A predictive mathematical model for the relationship between the amount dissolved in vitro at a particular time (eg, $Q_{60}$) or the time required for dissolution of a fixed amount (eg, $T_{50\%}$) and a summary parameter that characterizes the in vivo time course (eg, $C_{max}$ or AUC).
*Multiple Level C*: Predictive mathematical models for the relationships between the amount dissolved at several time points of the product and one or several pharmacokinetic parameters of interest.

Level A is the most informative and most useful from both scientific and regulatory perspectives in that it represents a point-to-point relationship between in vitro release and in vivo release/absorption from the dosage form. It can be used to predict the entire in vivo time course from the in vitro data. Multiple Level C is also useful as it provides the in vitro release profile of a dosage form with biological meaning. Level C can be useful in early stages of product development or setting meaningful specifications, although it does not reflect the complete shape of the plasma concentration-time curve. Level B utilizes the principles of statistical moment analysis. However, it is the least useful for regulatory applications because different in vitro or in vivo profiles may produce similar mean time values.

### 16.1.2 IVIVC and product development

The value of IVIVC in product development has been recognized since the early 1960s. Exploring in vitro/in vivo association or correlation is very useful in guiding formulation and process development. A validated IVIVC can support formulation and process changes and scale-up, help develop meaningful dissolution specifications, and support the use of dissolution as a surrogate for an in vivo study, since IVIVC provides a biological meaning to the results of the in vitro test.[2] Thus, availability of a validated predictive IVIVC can result in a significant positive impact on product quality, development efficiency, and reduced regulatory burden.

The approaches and challenges of developing an IVIVC have undergone extensive discussion and debate since the 1980s.[16−25] In general, there is increased uncertainty associated with developing an IVIVC for IR oral dosage forms because the in vivo apparent drug absorption is often a function of a multitude of variables, many of which are difficult to isolate or mimic in vitro. For example, a correlation between the in vitro dissolution and the in vivo absorption for IR dosage forms of highly water soluble drugs (eg, Biopharmaceutical Classification System 1 and 3) is usually not possible because gastric emptying or membrane permeation is often the rate-limiting step. Absorptive and efflux transporters and/or gut metabolism can play a significant role in the apparent absorption of BCS 2−4 compounds[26−28] depending on the dose, physicochemical, biopharmaceutical properties, and dissolution rate, making correlating dissolution with absorption difficult.[29] Compared to IR products, an IVIVC is more suitable for ER dosage forms where drug release is, by design, rate limiting in the absorption process. In addition, because a patient is typically exposed to a specific range of plasma levels over an extended period of time (eg, up to 24 hours) following administration of a modified-release (MR) dosage form, an in vitro test method with a qualitative or quantitative relationship with in vivo data is desired to assure the consistent in vivo performance. Hence, the FDA has recommended investigation of the possibility of an IVIVC in the development of ER dosage forms.[2]

## 16.2 DEVELOPMENT AND ASSESSMENT OF AN IVIVC

The regulatory guidance on IVIVC of ER oral dosage forms issued by the FDA in 1997 provides a comprehensive scientific framework and regulatory guideline to IVIVC model development, evaluation, and applications. In general, establishing an IVIVC consists of (1) study design, (2) model building, and (3) model validation based on an appropriate statistical assessment.

### 16.2.1 Study design and general considerations

Development of an IVIVC requires in vitro and in vivo data of formulations with varying in vitro release rates and corresponding in vivo differences. These data may come from studies at the early or late stage of product development, such as bioavailability studies conducted in the formulation screening stage or an in vivo study specifically designed to explore IVIVC.[11,30,31] The in vitro release rates, as measured by percent dissolved for each formulation studied, should differ adequately (eg, by 10%).[2]

To obtain useful data for IVIVC, discriminating dissolution methodology is essential. Based on the FDA

guidance,[2] in vitro data are preferably generated in an aqueous medium using USP apparatus I (basket) or II (paddle), operating within an appropriate range of rotation speeds (eg, 50−100 rpm). In other cases, USP apparatus III (reciprocating cylinder) or IV (flow-through cell) may also be used. Generally, any in vitro test method may be used to obtain the dissolution characteristics of the dosage forms as long as it is shown to be predictive of the in vivo performance. The dissolution profiles of at least 12 individual dosage units should be determined for IVIVC purposes. The coefficient of variation (CV) for the mean dissolution profiles of a single batch should be less than 20% at early time points and less than 10% at other time points.

According to the FDA guidance,[2] bioavailability studies for IVIVC development should be performed in humans with enough subjects in order to adequately characterize the absorption profiles of the drug product. Although crossover studies are preferred, parallel studies or cross-study analyses are also acceptable. The latter may involve normalization with a common reference treatment. The reference product in developing an IVIVC may be an intravenous solution, an aqueous oral solution, or an immediate-release product of the drug. In addition, IVIVCs are usually developed in the fasted state. When a drug is not tolerated in the fasted state, studies may be conducted in the fed state.

## 16.2.2 IVIVC modeling

The principles and methodologies of IVIVC modeling and assessment have been extensively addressed and reviewed in the literature.[32−34] Developing an IVIVC model begins with understanding the following mathematical principles for characterizing in vivo drug release/absorption profiles or parameters associated with different types of IVIVC.

### *16.2.2.1 Convolution and deconvolution approaches used in Level A correlation*

Convolution and deconvolution methods are essential tools for establishing Level A IVIVC. Convolution is a model-independent method based on linear system theory.

A linear system has the property that the response to a linear combination of inputs is the same linear combination of the individual responses (superposition). If the input is shifted in time by some amount, and the output is simply shifted by the same amount, such a system that is not sensitive to the time origin is known as a linear time-invariant (LTI) system.[35]

Once the impulse response function of a LTI system (ie, the way the system responds to a unit impulse) is measured, how the system will respond to any other possible inputs can be predicted in principle. This is because (1) the inputs to the system can be decomposed as a linear combination of some basic inputs, and (2) responses (or outputs) can be constructed as the same linear combination of the responses to each of the basic inputs in a wide variety of ways as illustrated in Fig. 16.1.

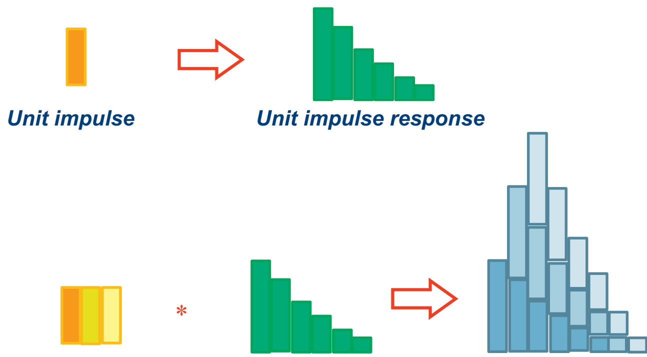



FIGURE 16.1 Illustration of input-response relationship of a LTI system (* denotes the convolution operation).

In pharmaceutical applications, drug disposition in the body is considered an LTI system. One of the measurable responses to the drug input (absorption) would be the plasma concentration-time profile. The response to an instantaneous (impulse) input would be the plasma concentration-time profile of following an intravenous bolus injection that is unique to individual drug molecules. Therefore, through applying the superposition principle of an LTI system in the study of the drug absorption process, a response, $C(t)$, to an arbitrary input, $f(t)$, of the system can be obtained using the following convolution integral[36]:

$$C(t) = f(t)^{*}C_{\delta}(t) = \int_{0}^{\infty} C_{\delta}(t-\tau)f(\tau)d\tau \tag{16.1}$$

where $C_{\delta}(t)$ is the unit impulse response (UIR) that defines the characteristic of the system. It is the response of the system to an instantaneous unit input, usually attainable from an IV bolus or oral solution. By the same principle, $f(t)$ can be obtained by deconvolution, the inverse operation of convolution. Their applications in IVIVC are illustrated in Fig. 16.2, and representative systems are provided in Table 16.1. The definition of a system is flexible and is determined by the nature of the time functions involved.[37] Depending on the specific $C_{\delta}(t)$ and input responses used to define a system, $f(t)$ obtained by deconvolution in IVIVC may represent the dissolution process, absorption process, or the combined processes of the two.

In exploring IVIVC, Level A correlation is usually estimated by a two-stage procedure, that is, deconvolution

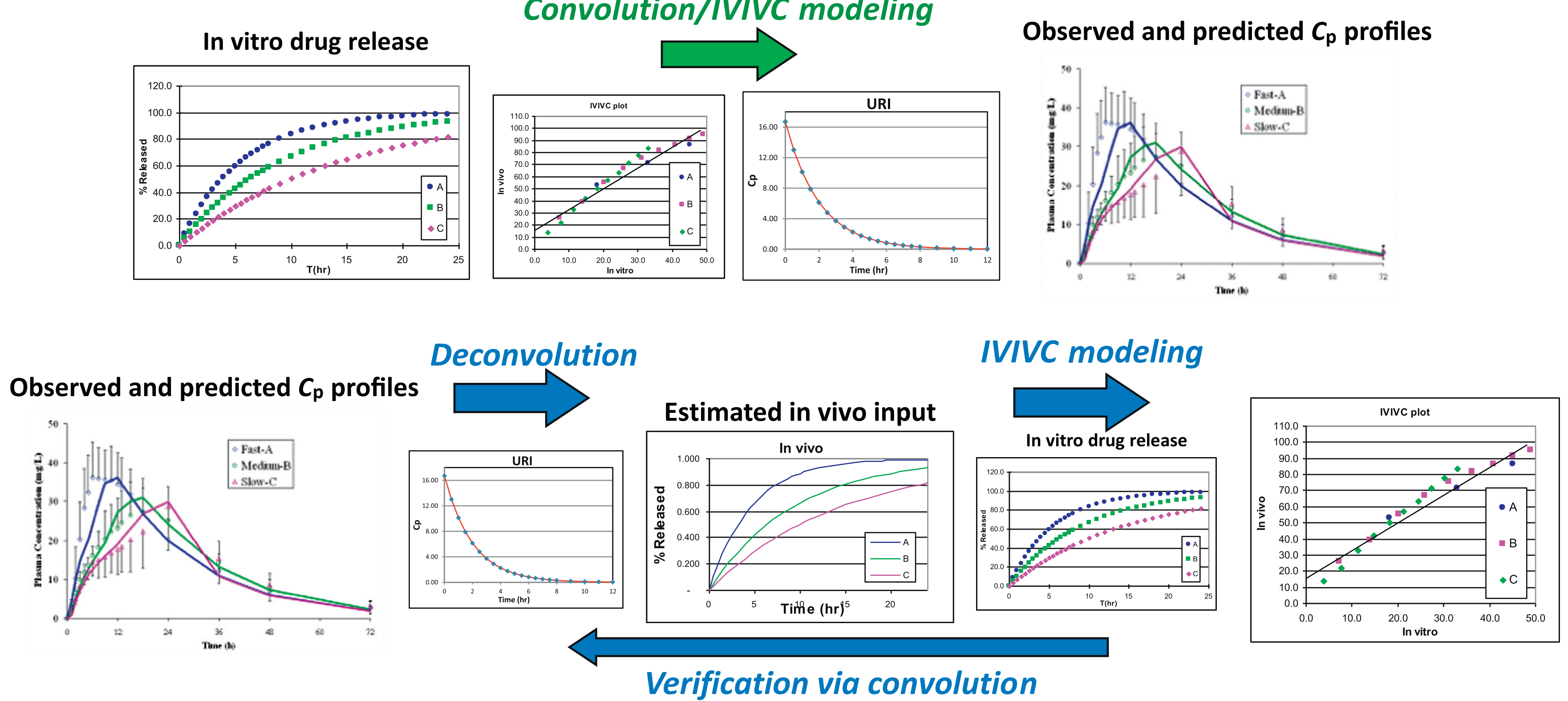



FIGURE 16.2 Illustration of convolution and deconvolution in IVIVC development.

TABLE 16.1 Illustration of System Definitions for Oral Administration

| Case | Unit impulse response $C_\delta(t)$ | Input response $C(t)$ | Input function $f(t)$ |
|---|---|---|---|
| I | Plasma level profile of IV bolus | Plasma level profile of oral solution | Absorption in the GIT |
| II | Plasma level profile of IV bolus | Plasma level profile of oral solid dosage form | Dissolution and absorption in the GIT |
| III | Plasma level profile of oral solution (or IR dosage form as an approximation) | Plasma level profile of oral solid dosage form | Dissolution in the GIT |



followed by correlating the fraction dissolved in vitro with the fraction released or absorbed in vivo. It may also be evaluated via a single-stage procedure, that is, a direct comparison of the observed with the predicted plasma concentration-time profiles obtained by convolution of the in vitro data and UIR. According to Eq. (16.1), the in vitro drug release and the in vivo input (release/absorption) estimated by deconvolution of the UIR with the observed plasma data are either directly superimposable or may be made to be superimposable by the use of a scaling factor when a 1:1 IVIVC exists. Similarly, the plasma concentration profile observed following oral administration should be in good agreement with that obtained by convolution of the UIR with the in vitro release data if there is a Level A IVIVC.

#### 16.2.2.1.1 General solution

The exact solution of convolution or deconvolution can be obtained by operation of Laplace transform if each functional form is defined:

$$L\{C(t)\} = L\{(C_\delta{}^* f)(t)\} = L\{C_\delta(t)\} L\{f(t)\} \tag{16.2}$$

$$f(t) = L^{-1}\{\bar{f}(s)\} = L^{-1}\left\{\frac{\bar{c}(s)}{\bar{c}_\delta(s)}\right\} \tag{16.3}$$

where $L$ and $L^{-1}$ denote Laplace transform and inverse Laplace transform, respectively. Deconvolution methods include explicit (numerical point-area and midpoint methods, least squares curve fitting using polyexponential, polynomial, spline functions) and implicit methods (prescribed function or deconvolution via convolution).[38–50]

Since the disposition of most drugs can be described by polyexponentials,

$$C_\delta(t) = \sum_{i=1}^{n} A_i e^{-\alpha_i t} \tag{16.4}$$

the in vivo input function $f(t)$ can be obtained using Eq. (16.3). For example, in the case of single-exponential disposition ($n = 1$), $C_\delta(t) = A_1 e^{-\alpha_1 t}$, and hence, $f(t)$, the input rate, is given by [40]:

$$f(t) = \frac{[C'(t) + \alpha_1 C(t)]}{A_1} \tag{16.5}$$

The amount of drug absorbed from time 0 to $t$, $X_a(t)$, is then obtained by integration:

$$X_a(t) = \int_0^t f(t)dt = \frac{\left[C(t) + \alpha_1 \int_0^t C(t)dt\right]}{A_1} \quad (16.6)$$

In cases where $C(t)$ or $f(t)$ cannot be fitted to an explicit function, numerical methods are used to deal with the raw data.

#### 16.2.2.1.2 Numerical deconvolution

It is well recognized that classical linear compartmental kinetic models exhibit superposition linearity due to their origin in linear differential equations. Hence, linear systems analysis, especially in the form of numerical algorithms, is conceptually simple, and thus it is a useful tool for assessing absorption and IVIVC. Deconvolution using purely numerical algorithms has been used for evaluation of IVIVC since the 1960s.[51–53] The general process for deconvolution usually uses the basic principle of deconvolution through convolution (DTC) to determine the input function. The DTC method is an iterative procedure consisting of three steps. The input function is first defined by the selection of its initial parameter values followed by convolution with UIR function to calculate the response (drug concentrations). Subsequently, the agreement between the observed drug concentrations and the calculated according to preset objective function is evaluated quantitatively. The iteration continues until the objective function reaches the preset values. According to Eq. (16.1), the response, $C(t)$, can be obtained given the UIR, $C_\delta(t)$, and the input, $f(t)$. Since the UIR defines the characteristic of the system, a general assumption is that the UIR would be identical for different formulations of the same compound. Therefore, explicit UIR is not required to calculate the response for one formulation $[C(t)_1]$ when another formulation has input $[f(t)_2]$ and response $[C(t)_2]$ data available.

The general numerical algorithms for convolution and deconvolution are shown as:

**Convolution $C(t) = f(t) * C_\delta(t)$**

$C(t)_1 = f(t)_1 * C_\delta(t)_1 * T$
$C(t)_2 = [f(t)_1 * C_\delta(t)_2 + f(t)_2 * C_\delta(t)_1] * T$
$C(t)_3 = [f(t)_1 * C_\delta(t)_3 + f(t)_2 * C_\delta(t)_2 + f(t)_3 * C_\delta(t)_1] * T$
$C(t)_n = [f(t)_1 * C_\delta(t)_n + f(t)_2 * C_\delta(t)_{n\text{-}1} + \cdots + f(t)_n * C_\delta(t)_1] * T$

**Deconvolution $f(t) = C(t)\ //\ C_\delta(t)$**

$f(t)_1 = (C(t)_1/T)/C_\delta(t)_1$
$f(t)_2 = (C(t)_2/T - f(t)_1 * C_\delta(t)_2)/C_\delta(t)_1$
$f(t)_3 = (C(t)_3/T - f(t)_1 * C_\delta(t)_3 - f(t)_2 * C_\delta(t)_2)/C_\delta(t)_1$
$f(t)_n = (C(t)_n/T - f(t)_1 * C_\delta(t)_n - f(t)_2 * C_\delta(t)_{n\text{-}1} - \cdots - f(t)_{n\text{-}1} * C_\delta(t)_2)/C_\delta(t)_1$

**Deconvolution $C_\delta(t) = C(t)\ //\ f(t)$**

$C_\delta(t)_1 = (C(t)_1/T)/f(t)_1$
$C_\delta(t)_2 = (C(t)_2/T - f(t)_2 * C_\delta(t)_1)/f(t)_1$
$C_\delta(t)_3 = (C(t)_3/T - f(t)_2 * C_\delta(t)_2 - f(t)_3 * C_\delta(t)_1)/f(t)_1$
$C_\delta(t)_n = (C(t)_n/T - f(t)_2 * C_\delta(t)_{n\text{-}1} - f(t)_3 * C_\delta(t)_{n\text{-}2} - \cdots - f(t)_n * C_\delta(t)_1)/f(t)_1$

The algorithms use piecewise integration to decompose Eq. (16.1). For example, the top block (convolution) uses pieces of the input and UIR functions, which are in reversed positions in the interval $0 \ldots t$ by assuming that both functions, input and UIR, are known as staircase functions, and both are given with a regular and common time interval, $T$, which requires all raw data points to be consistent. If this condition is not met, interpolations/extrapolations are needed to make the data consistent with $T$. The top block algorithm is most convenient for numerical calculations, especially when the inverse equations are employed in deconvolution, as shown in the midblock and the bottom block. Only for extremely small time intervals, all function values may be used just as points of the relevant time function. Otherwise it is essential to interpret them consistently as either "point" or "areas" representative for each time interval. Langenbucher presented an example dataset, which was used by several authors[44,46,54] to illustrate the convolution/deconvolution algorithms.[39] It is reproduced in Table 16.2 for reference. The data include the UIR, input, and response where $T$ is 0.5 or 1.0.

Based on the numerical algorithm, the data are used to illustrate the convolution and deconvolution calculations as shown in Table 16.3. The top block is the convolution calculation. The next two blocks are deconvolutions for calculating the inputs and UIR, respectively.

Although the numerical calculations are rather tedious, they are usually done by computer programs in actual applications. It is helpful to understand the underlying operations, especially when a customized program is needed. The commercial software commonly used to perform convolution and deconvolution calculations include IVIVC Toolkit for Phoenix, WinNonlin by Pharsight Corporation, and IVIVCPlus, an add-on module of GastroPlus by Simulations Plus, Inc. MS Excel has also been shown to be a useful tool for IVIVC applications.[55]

#### 16.2.2.1.3 Model-dependent deconvolution

Two commonly used deconvolution methods for estimating the apparent in vivo drug absorption profiles following oral administration of a dosage form are Wagner–Nelson and Loo–Riegelman methods.[56] These are model-dependent approaches based on mass balance. The Wagner–Nelson equation is derived from

**TABLE 16.2** An Example for the UIR, Input, and Response

| Time (h) | UIR | | | Input | | | | Response |
|---|---|---|---|---|---|---|---|---|
| | $C_\delta(t)$ | $C_\delta(t)_{0.5}$ | $C_\delta(t)_{1.0}$ | $f(t)$ | $f(t)_{0.5}$ | $f(t)_{1.0}$ | $\int f(t)dt$ | $C(t)$ |
| 0 | 20.227 | – | – | 0.41 | – | – | 0 | 0 |
| 0.5 | 16.364 | 18.296 | | 0.334 | 0.372 | | 0.185 | 3.36 |
| 1 | 13.549 | 14.957 | 16.888 | 0.272 | 0.303 | 0.341 | 0.336 | 5.487 |
| 1.5 | 11.481 | 12.515 | | 0.221 | 0.247 | | 0.459 | 6.774 |
| 2 | 9.946 | 10.714 | 11.748 | 0.18 | 0.202 | 0.227 | 0.56 | 7.492 |
| 2.5 | 8.792 | 9.368 | | 0.147 | 0.164 | | 0.641 | 7.831 |
| 3 | 7.91 | 8.351 | 8.928 | 0.119 | 0.134 | 0.151 | 0.708 | 7.921 |
| 3.5 | 7.225 | 7.568 | | 0.097 | 0.108 | | 0.762 | 7.851 |
| 4 | 6.681 | 6.953 | 7.296 | 0.079 | 0.088 | 0.099 | 0.806 | 7.681 |
| 4.5 | 6.239 | 6.46 | | 0.064 | 0.072 | | 0.842 | 7.452 |
| 5 | 5.873 | 6.056 | 6.277 | 0.052 | 0.058 | 0.066 | 0.871 | 7.191 |
| 5.5 | 5.561 | 5.717 | | 0.042 | 0.047 | | 0.895 | 6.916 |
| 6 | 5.29 | 5.426 | 5.582 | 0.035 | 0.039 | 0.044 | 0.915 | 6.639 |

*Reproduced from Cutler DJ. Numerical deconvolution by least squares: use of polynomials to represent the input function. J Pharmacokin Biopharm 1978;6:243–263.*

**TABLE 16.3** Illustration of the Numerical Algorithm for Convolution and Deconvolution Based on the Data in Table 16.2

| $\Delta t$ | Time | Calculation and calculated results | | True |
|---|---|---|---|---|
| 1 | 1 | $C(t)_1 = 0.341 \cdot 16.888 =$ | 5.759 | 5.487 |
| | 2 | $C(t)_2 = 0.341 \cdot 11.748 + 0.227 \cdot 16.888 =$ | 7.840 | 7.492 |
| 0.5 | 0.5 | $C(t)_1 = (0.372 \cdot 18.296) \cdot 0.5 =$ | 3.403 | 3.36 |
| | 1 | $C(t)_2 = (0.372 \cdot 14.957 + 0.303 \cdot 18.296) \cdot 0.5 =$ | 5.554 | 5.478 |
| | 1.5 | $C(t)_3 = (0.372 \cdot 12.515 + 0.303 \cdot 14.957 + 0.247 \cdot 18.296) \cdot 0.5 =$ | 6.853 | 6.774 |
| | 2 | $C(t)_4 = (0.372 \cdot 10.714 + 0.303 \cdot 12.5\ I\ 5 + 0.247 \cdot 14.957 + 0.202 \cdot 18.296) \cdot 0.5 =$ | 7.584 | 7.492 |
| 1 | 1 | $f(t)_1 = 5.487/16.888 =$ | 0.325 | 0.341 |
| | 2 | $f(t)_2 = (7.492 - 0.325 \cdot 11.748)/16.888 =$ | 0.218 | 0.227 |
| 0.5 | 0.5 | $f(t)_1 = 3.360/(18.296\ 0.5) =$ | 0.367 | 0.372 |
| | 1 | $f(t)_2 = (5.487 - 0.367 \cdot 14.957 \cdot 0.5)/(18.296 \cdot 0.5) =$ | 0.300 | 0.303 |
| | 1.5 | $f(t)_3 = (6.774 - 0.367 \cdot 12.515 \cdot 0.5 - 0.300 \cdot 14.957 \cdot 0.5)/(18.296 \cdot 0.5) =$ | 0.244 | 0.247 |
| | 2 | $f(t)_4 = (7.492 - 0.367 \cdot 10.714 \cdot 0.5 - 0.300 \cdot 12.515 \cdot 0.5 - 0.244 \cdot 14.957 \cdot 0.5)/(18.296 \cdot 0.5) =$ | 0.199 | 0.202 |
| 1 | 1 | $C_\delta(t)_1 = 5.487/0.341 =$ | 16.091 | 16.888 |
| | 2 | $C_\delta(t)_2 = (7.492 - 16.091 \cdot 0.227)/0.341 =$ | 11.259 | 1.1748 |
| 0.5 | 0.5 | $C_\delta(t)_1 = 3.360/(0.372 \cdot 0.5) =$ | 18.065 | 18.296 |
| | 1 | $C_\delta(t)_2 = (5.487 - 18.065 \cdot 0.303 \cdot 0.5)/(0.372 \cdot 0.5) =$ | 14.786 | 14.957 |
| | 1.5 | $C_\delta(t)_3 = (6.774 - 18.065 \cdot 0.247 \cdot 0.5 - 14.786 \cdot 0.303 \cdot 0.5)/(0.372 \cdot 0.5) =$ | 12.381 | 12.515 |
| | 2 | $C_\delta(t)_4 = (7.492 - 18.065 \cdot 0.202 \cdot 0.5 - 14.786 \cdot 0.247 \cdot 0.5 - 12.381 \cdot 0.303 \cdot 0.5)/(0.372 \cdot 0.5) =$ | 10.568 | 10.714 |

*Reproduced from Cutler DJ. Numerical deconvolution by least squares: use of polynomials to represent the input function. J Pharmacokin Biopharm 1978;6:243–263.*

a one-compartment model and the mass balance, $X_a = X_t + X_e$, where $X_a$, $X_t$, and $X_e$ are amounts of drug absorbed, in the body and eliminated at time $t$, respectively. By derivation, the amount of drug absorbed up to time $T$, $(X_a)_T$, is given by: $(Xa)_T = VC_T + kV\int_0^T Ctdt$, where $V$ is the volume of central compartment, $C_T$ is concentration of drug in the central compartment at time $T$, and $k$ is the first-order elimination rate constant. In the study of IVIVC, this is often expressed in terms of fraction ($F$) of the dose ($D$) absorbed for comparison with fraction released in vitro:

$$F_a(T) = \frac{(Xa)_T}{(Xa)_\infty} = \frac{C + k\int_0^T Ctdt}{k\int_0^\infty Ctdt} \tag{16.7}$$

where $F_a(T)$ or FD is the fraction of the bioavailable drug absorbed at time $T$. It should be noted that Eq. (16.7) is identical to Eq. (16.6). Therefore, the Wagner−Nelson method represents a special case of deconvolution with a single-exponential disposition. When intravenous data are not available, the apparent in vivo fractional absorption profile can be obtained by using terminal phase elimination rate constant, $k$, and partial areas under the plasma concentration curve using Eq. (16.7). However, it should be pointed out that: (1) $k$ value should be derived from the true elimination phase, which may be difficult for drugs with a prolonged absorption phase and/or long half-life; and (2) only apparent absorption is estimated using this method.

The approximate equation used in absorption analysis for the two-compartment model was first published by Loo and Riegelman in 1968.[57] Wagner published an Exact Loo−Riegelman method for a multicompartment model in 1983.[58] It is a general equation for the absorption analysis of one- to three-compartment models. It requires IV data for the calculation of absorption profiles. For biexponential disposition, mass balance leads to: $(X_a)_T = X_c + X_p + X_e$, where $X_c$ and $X_p$ are amounts of a drug in the central and peripheral compartments at time $T$, respectively. By derivation,[58] $(X_a)_T$ can be determined:

$$\frac{(Xa)_T}{Vc} = C_T + k_{12}e^{-k_{21}T}\int_0^T C_t e^{-k_{21}t}dt + k_{10}\int_0^T C_t dt \tag{16.8}$$

where $k_{12}$, $k_{21}$, and $k_{10}$ are the microconstants that define the rates of transport between compartments. On the basis of mass balance, $(X_a)_T = X_c + Xp_1 + Xp_2 + X_e$, a similar equation can be derived for triexponential disposition. The corresponding Exact Loo−Riegelman equations are given as:

$$\frac{(Xa)_T}{Vc} = C_T + k_{12}e^{-k_{21}T}\int_0^T C_t e^{-k_{21}t}dt + k_{13}e^{-k_{31}T}\int_0^T C_t e^{-k_{31}t}dt + k_{10}\int_0^T C_t dt \tag{16.9}$$

It can be shown that the Loo−Riegelman method is also a special case of deconvolution where in vivo disposition is described by two or three exponentials.[50] The theoretical and practical aspects of absorption analysis using model-dependent approaches have been thoroughly discussed by Wagner.[56]

#### *16.2.2.2 Mean time parameters used in Level B correlation*

Level B correlation is based on correlating mean time parameters that characterize the in vitro and in vivo time courses, for example, the in vitro or in vivo mean dissolution time (MDT) and in vivo MRT. Mean time parameters have been commonly utilized in pharmacokinetic studies and used to describe in vitro release. They are useful in studying specific models as well as less differentiated, more general system models. Many important concepts, definitions, and computations on this subject have been thoroughly discussed by Veng-Pedersen[59] and Podczeck.[60]

##### 16.2.2.2.1 In vivo parameters

By definition, MRT is the average total time a drug molecule spends in the introduced kinetic space. It depends on the site of input and the site of elimination. When the elimination of the molecule follows first-order kinetics, its MRT can be expressed by[59]:

$$\text{MRT} = \frac{\int_0^\infty tCtdt}{\int_0^\infty Ctdt} = \frac{\text{AUMC}}{\text{AUC}} \tag{16.10}$$

where AUMC is area under the moment curve. Estimates for MRT can be calculated by fitting $C(t)$ to a polyexponential equation followed by integration or by using trapezoidal rules.

For noninstantaneous input into a kinetic space, such as oral absorption, the MRT estimated from extravascular data includes a contribution of the mean transit time for input, known as mean absorption time (MAT, or mean arrival time, or mean input time).[59]

The MAT of drug molecules represents the average time taken to arrive in that space, and it can be estimated as:

$$\mathrm{MAT} = \frac{\int_0^\infty t f_{\mathrm{in}}(t)dt}{\int_0^\infty f_{\mathrm{in}}(t)dt} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \quad (16.11)$$

where $f_{in}(t)$ denotes an arbitrary rate of input into the kinetic space. For oral delivery, the MAT can be determined according to the equation:

$$\mathrm{MAT} = \mathrm{MRTpo} - \mathrm{MRTiv} \quad (16.12)$$

The term MAT thus obtained represents the mean transit time involved in apparent absorption process in the gastrointestinal (GI) tract. When the formulation contains a solid drug, the MAT includes in vivo dissolution as well as absorption. If data of the same drug given in a solution state are available, the in vivo MDT can be estimated by:

$$\mathrm{MDT}_{\mathrm{solid}} = \mathrm{MAT}_{\mathrm{solid}} - \mathrm{MAT}_{\mathrm{soln}} = \mathrm{MRT}_{\mathrm{solid}} - \mathrm{MRT}_{\mathrm{soln}} \quad (16.13)$$

#### 16.2.2.2.2 In vitro parameters

The measured amount of a drug substance in a cumulative-release profile can be considered as a probability that describes the time of residence of the drug substance in the dosage form. Therefore, a dissolution profile may be regarded as the distribution function of the residence times of each drug molecule in the formulation.[49] By definition, the MDT is the arithmetic mean value of any dissolution profile. If the amount of the drug remaining in the formulation is plotted as a function of time, the arithmetic mean value of the residence profile is the MRT of the drug molecules in the dosage form.

The techniques that are used to calculate MDT or MRT can be divided into model-independent (pragmatic plane geometry and prospective area) and model-dependent methods (eg, polyexponential, Weibull, and overlapping parabolic integration).[49] In general, model-independent approaches are used when release kinetics are unknown. These methods are based on area calculations from the amount released at various times. The following simple method is often used to determine the MDT and MRT using trapezoidal rules[49]:

$$\mathrm{MDT} = \frac{\int_0^\infty (M_{\max} - M(t))dt}{M_{\max}} = \frac{\mathrm{ABC}}{M_{\max}} \quad (16.14)$$

$$\mathrm{MRT} = \frac{\int_0^\infty tA(t)dt}{\int_0^\infty A(t)dt} \quad (16.15)$$

where ABC is the area between the drug dissolution curve and its asymptote. $A(t)$ is the amount of drug remaining in the dosage form at time $t$. $M(t)$ and $M_{max}$ are the amount of drug released at time $t$ and the maximal amount released, respectively. The model-dependent methods are based on the derived parameters of functions that describe the release profiles. It should be noted that one important source of errors in calculations comes from the often incomplete release. The calculation of the moments in such case is based on the maximum drug release. For systems that have a complete drug release, the size of errors depends on the number of data points and the curve shape.[49]

### *16.2.2.3 Summary parameters used in Level C correlation*

The extent and rate of drug release from a dosage form are often characterized by one or more of the single measurements (eg, $Q_{60}$, $T_{50\%}$, or $T_{85\%}$), particularly when there are not enough data points available to define the time functions of the profiles, or there are simply no suitable models that describe the dissolution curves. These parameters are most often obtained either directly from the dissolution measurements or by interpolation. Although they do not adequately characterize the whole dissolution process, they are utilized in quality control and in Level C correlation studies. The in vivo parameters used to correlate with the in vitro parameters are bioavailability parameters reflecting the rate and extent of absorption (eg, AUC and $C_{max}$).

### *16.2.2.4 Establishment of a Level A IVIVC model*

Establishing an IVIVC model requires in vitro data from formulations with different release rates (eg, fast, medium, and slow) and a discriminating in vitro test methodology. The corresponding in vivo response can be plasma concentrations or the amount of drug released and/or absorbed in vivo. The latter is obtained from the observed plasma concentration-time curve by deconvolution. There are advantages and disadvantages for either type of response variable. When plasma concentration is used as a response variable (single-stage approach), the link between the in vitro dissolution profile with the in vivo plasma concentration profile has clear clinical relevance because many pharmacokinetic parameters such as $C_{max}$, $T_{max}$, and

AUC are directly derived from the plasma concentration-time profile. Using the amount of drug released/absorbed as a response variable (two-stage approach) is intuitively straightforward because the in vitro and in vivo parameters are directly compared.

### 16.2.2.4.1 Two-stage approach

A deconvolution-based IVIVC model is established using a two-stage approach that involves an estimation of the in vivo release/absorption profile from the plasma concentration-time data using an appropriate deconvolution technique (eg, Wagner–Nelson, numerical deconvolution) for each formulation. Subsequently, the calculated in vivo percent absorbed or released is correlated with the percent released in vitro, as illustrated in Fig. 16.3 using a basic linear model with intercept ($a$) and slope ($b$):

$$(\%\ \text{absorbed})_{\text{in vivo}} = a + b(\%\ \text{released})_{\text{in vitro}} \quad (16.16)$$

A slope closer to one indicates a 1:1 correlation, and a negative intercept implies that the in vivo process lags behind the in vitro dissolution. A positive intercept has no clear physiological meaning. It can be a result of relatively high variability or curvature at the early time points. When the in vitro data are not in the same time scale as the in vivo absorption, it is usually necessary to incorporate a scaling factor, such as time-shifting and time-scaling parameters, within the model. Nonlinear models, while uncommon, may also be appropriate.[13] The two-stage approach is the most frequently used in building IVIVC models.

For an IVIVC with a two-stage approach, an important aspect is to find the relationship between the in vitro dissolution and in vivo dissolution/absorption profiles. The general strategy is to use scaling and/or shifting or have the time scaled and shifted to match the in vitro and in vivo profiles. In the software commonly used, such as Winnonlin Phoenix, the following models are built in.

**A.** Fabs = Diss(Tvivo)
**B.** Fabs = AbsScale * Diss(Tscale * Tvivo)
**C.** Fabs = AbsScale * Diss(Tscale * Tvivo − Tshift)
**D.** Fabs = AbsScale * (Diss(Tscale * Tvivo − Tshift) − AbsBase)

Where Fabs is the in vivo absorption fraction; Diss() is a function for dissolution, dependent on the in vitro time in the parenthesis, which could be the same as in vivo time: Tvivo as in model A, the scaled in vivo time by Tscale as in B, or the in vivo time scaled by Tscale and shifted by Tshift as in C and D. In B, C, and D, to get the in vivo fraction (Fabs), the in vitro dissolution (Diss) has to be scaled by AbsScale. Before this scaling, in D, Diss would be corrected by baseline (AbsBase).

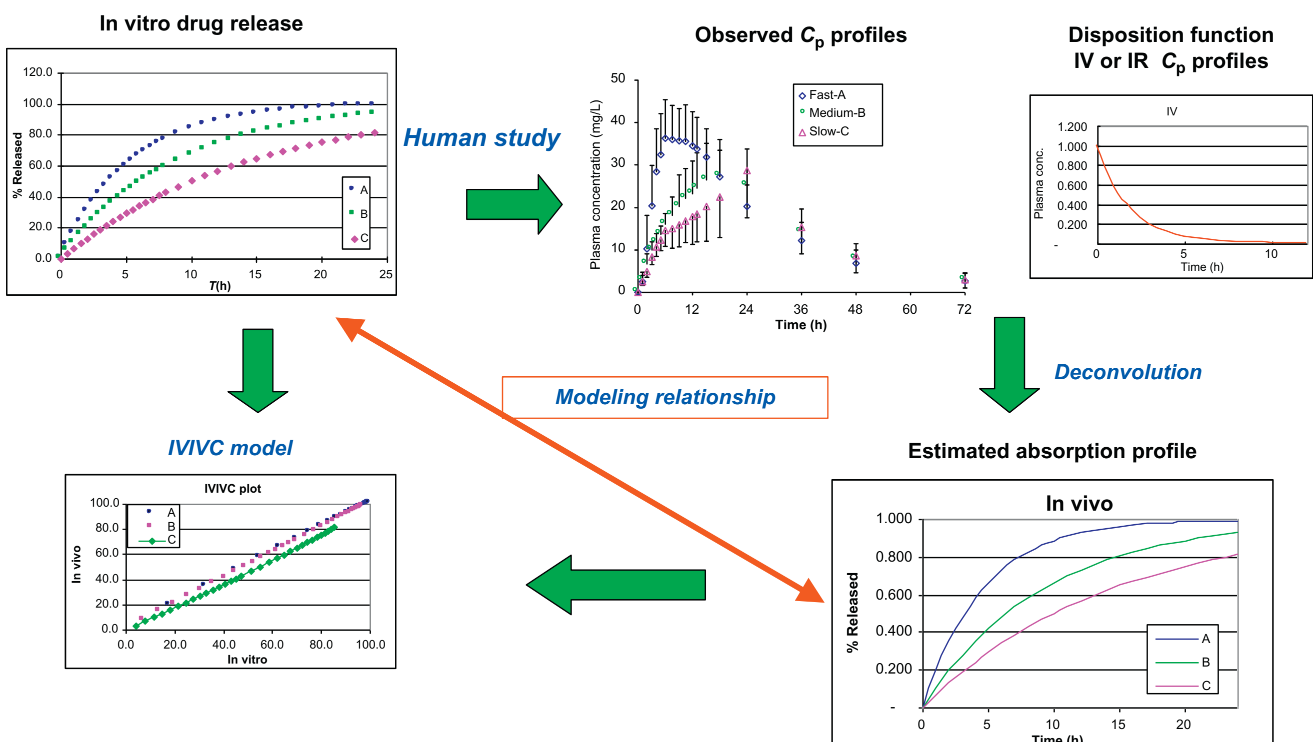



FIGURE 16.3 Illustration: building a Level A IVIVC model using the two-stage approach.

When all the scaling and shifting factors are included, it may be difficult to identify the appropriate model. To get an insight of the effect of these factors, a simulation study was performed in which the effects of three levels of each of the scaling (scale, AbsScale in B, C and D), baseline correction (shift, AbsBase in D), time scaling (Tscale), and time shifting (Tshift) were examined. The levels for the four parameters are provided below:

| Level | Scale | Shift | Tscale | Tshift |
|---|---|---|---|---|
| 1 | 0.3 | −30 | 0.5 | −5 |
| 2 | 1 | 0 | 1 | 0 |
| 3 | 1.5 | 30 | 2 | 5 |

There were a total of 81 possible combinations, and the results are shown in Fig. 16.4, where the values of the four factors are labeled in each panel.

Panel 41 shows case A, where the in vitro time is the same as in vivo time, resulting in a 1:1 relationship. The in vitro and in vivo profiles are superimposed. When the model is baseline corrected, the shape of the profiles does not change, and the profile just shifts up (or down), while scaling gets an $n$:1 relationship ($n$ is dependent on the values of AbsScale). The time scaling and shifting lead to the inconsistent in vitro time and in vivo time and different shapes of the profiles. Time scaling and shifting are needed if the in vitro and in vivo profiles exhibit different rates and/or a difference in lag time.

In addition to these built-in models, the software usually allows the user to specify a customized model. As shown in Fig. 16.5, the relationship between the in vivo and in vitro profiles seems to follow a sigmoidal shape, and an $E_{max}$ model could be used.

In summary, the patterns of in vitro and in vivo profiles may provide clues for an appropriate model. Plotting the in vitro and in vivo data at the same time points is helpful for recognition of the patterns in addition to the Levy plots.

#### 16.2.2.4.2 Single-stage approach

An alternative modeling approach based on convolution can be utilized to directly predict the time course of plasma concentrations using Eq. (16.1) in a single step.[61] Based on the assumption of equal or similar release rates between in vitro and in vivo, the input rate, $f(t)$, is modeled as a function of the in vitro release data with or without time scaling to predict the in vivo plasma profiles by convolution with the dose-normalized plasma data from an IV or IR reference dose. The IVIVC is assessed and validated by statistically comparing the predicted with the observed plasma levels. This convolution-based modeling focuses on the ability to predict measured quantities rather than indirectly estimated in vivo fraction absorbed and/or released. Thus, the results are more readily evaluated in terms of the effect of in vitro release on in vivo performances, for example, AUC, $C_{max,}$ and duration above minimum effective concentrations. For instance, in using this approach to estimate the plasma concentrations from the in vitro data, a poly-exponential UIR with lag time could be used in the model as follows:

$$C_\delta(t) = \sum_{i=1}^{nex} A_i{}^* e^{-\alpha_{(i)}(t-t_{lag})} \qquad (16.17)$$

where, $nex$ is the number of exponential terms in the model, $t_{lag}$ is the absorption lag time, and $C_t$ is the plasma concentration at time $t$. The input rate may be modeled as a function of the in vitro cumulative amount dissolved. For example, Veng-Pedersen, et al. reported a scaled convolution-based IVIVC approach by which the dissolution rate curve was first obtained via differentiation of a monotonic quadratic spline fitted to the dissolution data. Using time and magnitude scaling, the dissolution curve was then mapped into a drug concentration curve via a convolution by a single exponential and the estimated UIR function. The model was tested by cross-validation and demonstrated to be predictive of the systemic drug concentration profiles from the in vitro release dissolution data using four different tablet formulations of carbamazepine.[62]

It should be noted that the single-stage approach is based on the assumption of an LTI relationship between the input (drug release) and the response (plasma concentrations). Multiple formulations with different release rates are usually used in establishing an IVIVC. If a significant fraction of the dose of the slow-releasing formulations is released beyond the site (s) of drug absorption (ie, truncated absorption), an overestimation of plasma concentrations can occur because Eq. 16.1 predicts the same dose-normalized AUC as the reference dose used to estimate $C_\delta(t)$.[62] To address the potential discrepancies between in vitro and in vivo release/absorption, Gillespie proposed an extended convolution-based IVIVC model using a function relating the cumulative amounts released or the release rate in vitro ($x_{vitro}$) to that in vivo ($x_{vivo}$), $x_{vivo} = f\ (x_{vitro})$. Thus, plasma concentrations of multiple ER formulations can be more accurately predicted by substituting $f(t)$ with $x_{vivo}$ in Eq. (16.1) if there is an IVIVC. Selection of a specific functional form of $x_{vivo}$ can be based on a mechanistically understanding of the in vitro/in vivo relationship or semiempirically based on the goodness of model fitting. Certain plausible relationships include linear, nonlinear, or

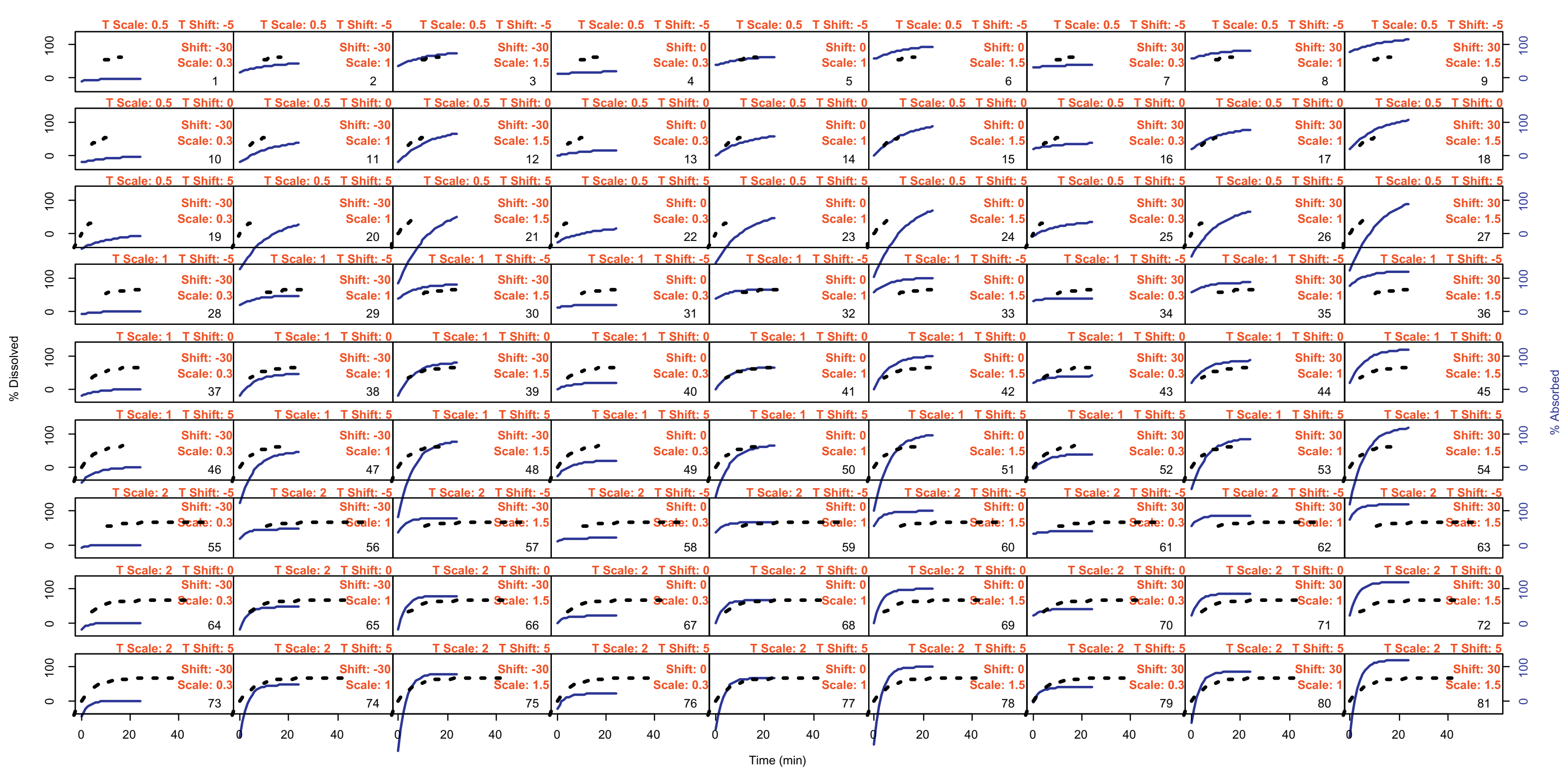


FIGURE 16.4 Simulated in vitro and in vivo profiles based on Model D Fabs = AbsScale * (Diss(Tscale *Tvivo − Tshift) − AbsBase) (dashed and solid lines are the in vitro dissolution and in vivo absorption profiles).

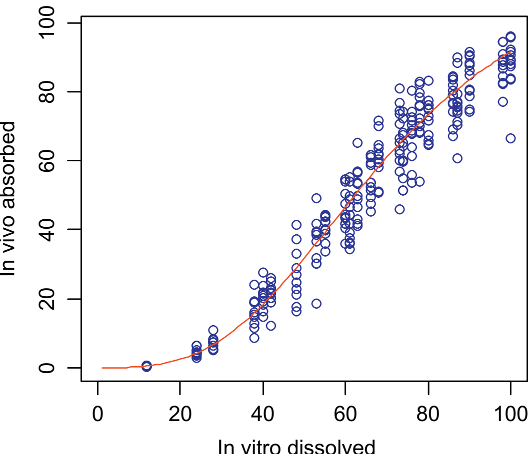



FIGURE 16.5 A nonlinear in vitro/in vivo relationship fit by $E_{max}$ model.

time-variant functions; linear or nonlinear time-scaling for taking into account the effects of lag time; truncated absorption; or saturable presystemic metabolism.[62,63] For ER systems, the apparent absorption in vivo is limited by the drug release from the dosage form, of which the kinetics is determined by the system design. In many cases, the release kinetics can be described by one of the following models: zero-order, first-order, square root of time, or Peppas's exponent models.[64] Therefore, the parametric function, $C(t)$, describing the plasma concentrations of an ER dosage form can be defined via convolution of $C_\delta(t)$ with the input, $f(t)$, prescribed from the in vitro model according to Eq. (16.1). For instance, the functional form of $C(t)$ can first be obtained by convolution of a prescribed function of input (eg, first-order) with a known UIR. The unknown parameters remaining in the $C(t)$ equation are those from the prescribed input function, $f(t)$, which can then be solved by fitting the $C(t)$ equation to the observed plasma concentrations. The resulting $f(t)$ is compared with the observed in vitro data to evaluate IVIVC. This approach is also known as DTC, as discussed in the previous section.

In predicting $C(t)$ by convolution, data from an IV or oral solution is desirable because it provides an estimate of $C_\delta(t)$ independent of the ER data. However, such a reference dose is not always available, particularly for compounds having low aqueous solubility. Nevertheless, an estimation of $C(t)$ by convolution for evaluation of IVIVC is still possible using only data from ER formulations.[62] In such cases, the prescribed parametric functional forms of both $C_\delta(t)$ and $f(t)$ can be mechanistically or empirically selected and substituted into Eq. (16.1). The parameters of $C_\delta(t)$ are then estimated by fitting the overall convolution model to the plasma concentrations of ER formulations. Predictive performance of the IVIVC is evaluated by comparing the predicted and observed results. It should be pointed out that the ability of the model to predict changes of the in vivo plasma concentrations with varying release rates should be validated by separately or simultaneously fitting the data from multiple formulations. By doing so, a $C_\delta(t)$ function can be reliably defined. Thus, one of the most critical requirements of this approach is to use at least two ER formulations with different release rates in the assessment of IVIVC.[61]

While the use of the two-stage procedure is more widespread, the convolution approach has gained increased interest. O'Hara et al. compared odds and identity models that include a convolution step using the data sets of two different products and a nonlinear mixed-effects model fitting software to circumvent the unstable deconvolution problem of the two-stage approach.[65] Gaynor et al. used a simulation study to show the convolution modeling approach produces more accurate results in predicting the observed plasma concentration-time profile.[66] Jacobs et al. described an IVIVC model for an oral product consisting of IR and ER components of galantamine by combining the IR and ER pharmacokinetic profiles using a one-stage convolution-based method.[67] The average percentage prediction error (PE) indicated a good fit of the new model.

#### 16.2.2.4.3 Compartmental and population approach

Both convolution- and deconvolution-based methods assume that the system being modeled is linear, but in practice, this is not always the case, as many drugs are eliminated by nonlinear (saturable) processes.[68–70] Therefore, a linear system approach can fail when the disposition of a drug substance falls within a nonlinear range. The extent of this failure depends on the magnitude of the nonlinearity observed.[71] To overcome problems resulting from nonlinear kinetics, the use of a method that can reliably accommodate the nonlinear characteristic is imperative. One of the effective methods to analyze nonlinear data is to use a compartmental approach. In a published study, the performance of a compartmental approach for nonlinear systems using the population approach for IVIVC modeling is evaluated.[72]

The population approach is to seek the sources and correlation of the variability in vivo, in vitro, and their relationship. The principles are similar to population pharmacokinetics (popPK), and understanding of the basic popPK concept will be greatly helpful. Fig. 16.6 introduces the concept of two types of variability considered by popPK. The solid circles are the data points for subject 1, from which a fit line is generated (dashed line). The open circles are the data points for all other

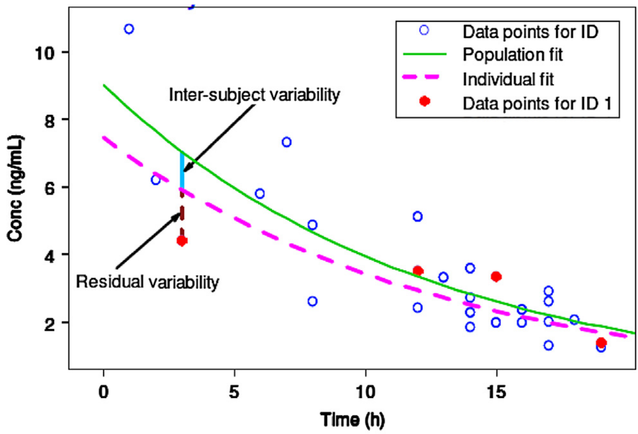



FIGURE 16.6 An illustration of popPK concept.

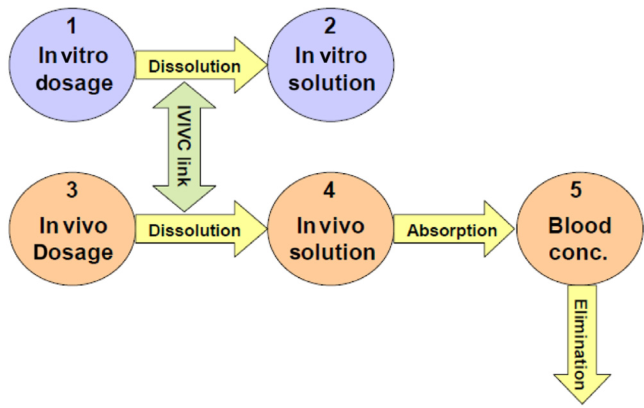



FIGURE 16.8 IVIVC based on a compartmental approach.

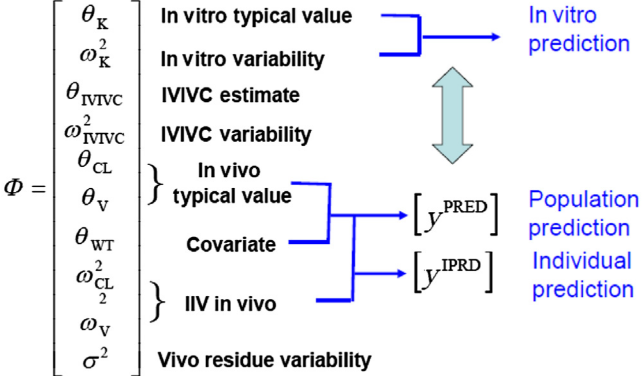



FIGURE 16.7 Various sources of variability considered in population IVIVC.

subjects. A population fit line (solid line) is generated from all the points (both solid and open circles). If one data point at a specific time (3.5 hours) for a specific subject (ID 1) is considered, conceptually, the PE is composed of two parts: one resulting from interindividual variability and another from residual variability.

For population IVIVC, not only the residue variability and the intersubject variability but also the in vitro variability and the variability of in vitro/in vivo correlation should be considered, as shown in Fig. 16.7. Clearance (CL) and volume distribution (V) are different from their typical values ($\theta_{CL}$ and $\theta_V$) due to the fixed effects such as weight ($\theta_{WT}$), age ($\theta_{AGE}$), and gender ($\theta_{SEX}$). They also can be affected by the intersubject variability ($\omega_{CL}^2$ and $\omega_V^2$) and the residue variability ($\sigma^2$). In addition, the fixed effect ($\theta_K$) and the random effect ($\omega_K^2$) for in vitro (dissolution) performance and the fixed effect ($\theta_{IVIVC}$) and the random effect ($\omega_{IVIVC}^2$) for the correlation should be taken into consideration.

Fig. 16.8 shows how an IVIVC relating to in vitro and in vivo drug dissolution could be described in terms of compartments.[73] This technique retains most of the benefits of the convolution approach: in vitro fractions dissolved and in vivo plasma concentrations are modeled directly in a one-step process, and the IVIVC can be specified to incorporate random effects, time dependence, and scale factors as required by each particular set of data.

#### *16.2.2.5 Establishment of a Level C IVIVC model*

Building a Level C IVIVC model is rather straightforward. It involves correlating the amount dissolved at various time points with $C_{max}$, AUC, or other suitable bioavailability parameters. Data from at least three formulations of different dissolution rates are required for establishing a linear or nonlinear relationship between the in vitro and in vivo parameters because each data point of the correlation plot corresponds to only one formulation. A single-point Level C correlation may facilitate formulation development or allow a dissolution specification to be set at the specified time point. The information is generally insufficient for justifying a waiver of a bioequivalence study. A multiple-point Level C correlation may be useful to support a biowaiver if the correlation has been established over the entire dissolution profile with one or more bioavailability parameters of interest. A relationship should be demonstrated at each time point with the same parameter such that the effect on the in vivo performance of any change in dissolution can be assessed. When such a multiple Level C correlation is achievable, the development of a Level A correlation is often more likely. A multiple Level C correlation should be based on at least three dissolution time points covering the early, middle, and late stages of the dissolution profile.[2]

### 16.2.3 Evaluation of a correlation

To assure a useful and reliable Level A IVIVC, the FDA guidance requires that the model should be demonstrated consistently with two or more formulations with different release rates. Therefore, the first important step in evaluating the appropriateness of the model is to test whether a single correlation fits all tested formulations. One of the statistical assessment approaches is to compare the fit of a reduced model where all tested formulations are fitted to a single correlation line with that of a full model, where each formulation is fitted to a different correlation line.[33] If both models fit well, the IVIVC is considered validated. If the full model fits well, but the reduced model does not, or if the full model is statistically different from the reduced model at significance level of 0.05, the IVIVC becomes formulation dependent and, therefore, is invalid. It is noted that the time-scaling factor, if used, should also be the same for all formulations. Different time scales for each formulation indicate the absence of an IVIVC.[2]

Following the establishment of a Level A IVIVC model, it is necessary to demonstrate that prediction of the in vivo performance from the in vitro dissolution characteristics is accurate and consistent. The FDA guidance suggests evaluating the goodness of fit by measuring the prediction error (PE), that is, differences between the observed and the predicted values over a range of in vitro release rates. As illustrated in Fig. 16.9, determination of PE involves calculation of the in vivo absorption (input) profiles from the in vitro data using the established IVIVC model, followed by the prediction of the corresponding plasma concentration profiles via convolution. The guidance further elaborates approaches to validate the model internally and externally. Internal validation can be accomplished through measuring PE using data from the same study used to develop the IVIVC. The internal PE evaluates how well the model describes the data used to develop the IVIVC. It could be adopted for cases where the IVIVC was derived using two or more formulations with different release rates, providing the drug is not considered a narrow therapeutic index drug. The external validation approach requires a data set that was not used in the development of the IVIVC, such as formulations with a different release rate, formulations with minor manufacturing process changes, or a formulation from a different manufacturing batch obtained from a different study. It is desirable and affords greater confidence in the model.

The criteria used in the FDA guidance on IVIVC are as follows: For predicted $C_{max}$ and AUC, the mean absolute percent prediction error (% PE) should not exceed 10%, and the PE for individual formulations should not exceed 15%. A PE of 10–20% indicates inconclusive predictability and illustrates the need for further study using additional data sets. For drugs

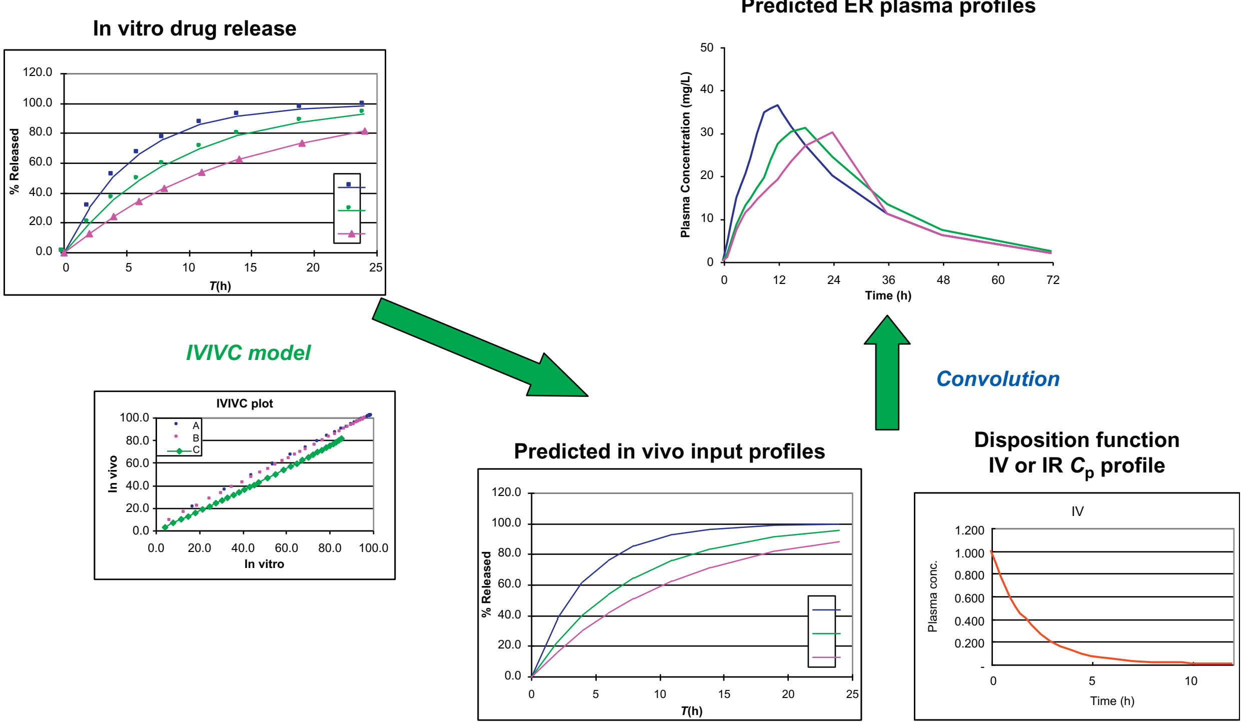



FIGURE 16.9 Illustration: prediction in IVIVC model validations and applications.

with a narrow therapeutic index, external validation is required despite acceptable internal validation, whereas internal validation is usually sufficient with nonnarrow therapeutic index drugs. In general, the less data available for the initial IVIVC development and evaluation of predictability, the more additional data may be needed to define completely the IVIVC's predictability. A combination of three or more formulations with different release rates is considered optimal.[2]

For Level C correlations, assessment of the predictability will depend on the type of application for which the correlation is to be used. The methods and criteria are the same as those for a Level A correlation. A recent example of the successful development and validation of a Level C IVIVC was reported by Kesisoglou et al. for suvorexant IR tablets based on amorphous solid dispersion.[74] Four different batches of tablets were manufactured using a hot-melt extrusion process and compressed at different tablet hardnesses to produce distinct dissolution profiles. These batches were evaluated in a relative bioavailability study in healthy volunteers followed by investigation of a relationship between dissolution and $C_{max}$. A validated multiple Level C IVIVC was developed between $C_{max}$ and $Q_{10}$, $Q_{15}$, $Q_{20}$, $Q_{30}$, $Q_{45}$ and the disintegration time, and it was used to set clinically relevant dissolution specification and in process control of tablet hardness.

## 16.3 CONSIDERATIONS IN IVIVC DEVELOPMENT

Defining a quantitative and reliable relationship between in vitro drug release and in vivo absorption is highly desired for the rational development, optimization, and evaluation of solid dosage forms and the manufacturing process. A validated IVIVC can significantly (1) increase the development efficiency by reducing the time and resources required for formulation and process development, scale-up, and optimization; (2) assure product quality by setting meaningful specifications; and (3) reduce regulatory burdens by using an in vitro test as a surrogate to in vivo bioavailability studies required for certain postapproval changes. Therefore, exploring and developing IVIVC where possible should be an important part of solid oral product development.

Since the early 1990s, extensive colloquiums and research publications have mainly focused on the basic principles, development methodology, modeling, evaluation, and applications of IVIVC and have laid a solid scientific foundation for regulatory guidance and decisions. Most of the relevant scientific discussions are based on the premise of availability of in vitro and in vivo information appropriate for establishing an IVIVC. However, it should be emphasized that modeling and statistical assessment is only one aspect of IVIVC development, and obtaining suitable in vitro and in vivo data is not a given. To achieve an IVIVC, at least two formulations that differ in the in vivo and in vitro performance should be available. In fact, many failed attempts in achieving an IVIVC for solid products can usually be attributed to a lack of a predictive in vitro test or an adequate in vivo difference between test formulations. Thus, it is crucial to understand how a drug's physicochemical and biopharmaceutical properties, delivery technology, and formulation design and their interplays with both the gastrointestinal tract (GIT) and the in vitro test methodology may impact in vitro and in vivo data.

### 16.3.1 In vivo absorption versus in vitro test considerations

In developing IVIVC, the in vitro parameter commonly utilized is drug release, which can be determined with precision under a controlled condition. It is a function of drug and dosage form characteristics and test methods and conditions. The in vivo response is usually the dissolution and absorption estimated from the availability of the drug in systemic circulation that typically has high variability. It is a function of a multitude of physicochemical, biopharmaceutical, and physiological variables, formulation, and their interactions. For test formulations that exhibit varying apparent in vivo absorption characteristics, the most critical element in establishing an IVIVC is the ability of in vitro tests to correlate quantitatively with the in vivo performance. To assess the challenges and the opportunities of developing a predictive and meaningful in vitro test, it is essential to first understand the absorption characteristics of the drug substance and the complexity of drug absorption from dosage forms in the GI tract.

#### *16.3.1.1 Apparent drug absorption from the GI tract*

The in vivo drug release and subsequent absorption from a solid product, particularly a modified-release dosage form, take place in one of the most complex environments where the interplays of the GI tract with the dosage forms as well as with intralumenally released drugs is highly dynamic. The process is known to be influenced by (1) drug property (solubility, ionization, stability, solid phase, lipophilicity, permeability, surface area, and wetting property); (2) dosage form design (dose, release mechanism, composition, type,

size and transit characteristics of the dose unit, sensitivity to shear force, and drug release location or duration); and (3) GI physiology and biology (motility, residence time, food, lumen contents, fluid volume, transport pathways and mechanism, enterohepatic recycling, effective permeability, surface area, metabolism, uptake and efflux transporters, and microflora). This type of information is highly valuable in anticipating and deciphering in vitro and in vivo data of the drug molecule and the dosage forms as related to the development of an IVIVC. For example, an apparently truncated absorption or a significantly decreased extent or rate observed with the slow-release formulations 4−5 hours post dosing may be related to one or more the following factors discussed in chapter "Rational Design of Oral Modified-Release Drug Delivery Systems" and literature[75−81]: lower apparent permeability in the large bowel due to inadequate lipophilicity or involvement of gut enzymes/transporters; high dose/solubility ratio; microbiotic degradation or decreased delivery rate of the formulations due to a change in lumenal environment. When assessing discrepancies between in vitro and in vivo data, delivery technology and formulation design, and release mechanism, in vitro test method and condition are important factors to consider. For instance, a dosage form generally experiences a low and well-controlled flow field and shear rate in a typical in vitro test, while it is known to be subjected to a myriad of physical forces in vivo, including pressure and shear stress from cyclic strain associated with villous motility and repetitive deformation engendered by peristaltic muscular contractions and relaxation of the intestinal wall.[82] As a result, it is not uncommon to observe differences between in vitro and in vivo behaviors for formulations that are sensitive to the release environment, particularly for hydrophilic matrix formulations containing a relatively high percentage of soluble components and/or a low percentage of low-viscosity-grade polymers. For example, an appreciably more rapid in vivo release and/or increased in vivo differentiation among formulations exhibiting different in vitro release rates can be attributed to a change of the release-control mechanisms from diffusion in vitro and erosion in vivo because a predominantly diffusion-controlled drug release in vitro is known to be less sensitive to variations in gel strength and matrix integrity.[11,12,83,84] On the other hand, decreased in vivo discrimination can be a result of a weaker and more environmentally sensitive gel, active pharmaceutical ingredient (API)−polymer interaction, or nonnegligible contribution of particle dissolution to the drug release in vivo due to a nonsink condition.[85] More detailed information on variables that may influence apparent absorption, including drug properties, biopharmaceutical factors, and product/process design, can be found in chapters "Oral Absorption Basics: Pathways and Physicochemical and Biological Factors Affecting Absorption," "Oral Drug Absorption: Evaluation and Prediction," "Predictive Biopharmaceutics and Pharmacokinetics: Modeling and Simulation," "Rational Design of Oral Modified-Release Drug Delivery Systems," and "Product and Process Development of Solid Oral Dosage Forms," respectively.

#### 16.3.1.2 *In vitro test method*

Among various in vitro physicochemical tests, dissolution testing is one of the most important tools for product quality assessment, process control, and for assuring sameness after making formulation or process changes. While different dissolution tests have been applied to determine drug release, including the commonly used compendia methods (eg, basket, paddle, or reciprocating cylinder), the dissolution rate of a specific dosage form is, in many cases, an arbitrary parameter that often varies with the test methodology with respect to its relevance to the in vivo performance unless an IVIVC is demonstrated.[32,86] One of the main reasons is that none of the existing in vitro methods represents or mimics the highly dynamic and complex in vivo conditions and a wide range of variables involved in the drug absorption from solid dosage forms in the GI tract.

Over the years, the exploration and the development of various in vitro tests or models have been undertaken in an attempt to match or predict in vivo data and/or to simulate specific aspects of the GI condition.[87] These studies include designs ranging from simple and very complex setups and can be divided into three broad categories listed in Table 16.4. The common attempt made in these studies is to match in vivo data by adjusting the in vitro discrimination and release rate of different test formulations through altering test media, hydrodynamics, mixing, creating shear force, or dynamic flows. It should be noted that many tests are static and only capable of imitating very limited aspects of the complex in vivo conditions. For example, some test methods are focused on test medium, supersaturation/ precipitation, and/or apparatus variables. Other models based on multiple compartments or vessels are designed to integrate dissolution with digestion and absorption or simulate motility and successive dynamic processes occurring in the GI tract. The most complicated in vitro model is a multicompartment, dynamic, computer-controlled system designed to simulate the human stomach, small intestine (TIM-1), and large intestine (TIM-2), respectively.[88,89] The system is intended for gathering information about the effects of various simulated GI conditions on biopharmaceutical behavior of the API

**TABLE 16.4** Types of In Vitro Drug Release Tests for Predicting In Vivo Performance

| Type | Examples | Variables altered | References |
|---|---|---|---|
| Standard pharmacopoeial methods | USP I, II, III, or IV | Hydrodynamics; agitation; pH; ionic strength; surfactants; enzymes; additives (eg, bile salts, lecithin, fat, milk) | 10,93–99 |
| Modified pharmacopoeial methods | USP II + polystyrene beads<br>USP II + Stationary basket<br>USP II + two-phase<br>Milk/fat as test medium<br>FeSSIF/FaSSIF as test medium<br>Ex vivo intestinal fluid | Hydrodynamics; shear stress; mechanical attrition; mixing; pH; test medium; release vs dissolution, etc. | 100–112 |
| Complex models | Rotating dialysis cell<br>Flow-through cell drop method<br>Multicompartmental systems | pH; food; mixing; motility; transit; digestion, secretion; microflora, permeation, etc. | 8,86,113–119 |

and dosage forms. However, setting up experiments and generating data are time consuming and extremely labor intensive. It is unsuitable for routine product quality control. In addition, literature reports of its utility and application in IVIVC development are still absent, although there are reports of its use in evaluating relative formulation performance.[90,91]

In summary, the most important aspect of IVIVC is to develop a predictive in vitro test. In practice, product quality and performance characterized by such a test is the drug release from the dosage forms. However, the state of the art has yet to allow the development of a universal predictive model independent of drug molecule and dosage form characteristics because physiological, physiochemical, and biological conditions of the GI tract and their interplays with the API and drug products in vivo are extremely complex[7,85,92] Nevertheless, it remains possible to develop tailored predictive tests for an individual API and/or product based on the understanding of the critical aspects of importance to in vivo absorption. For instance, when in vivo drug release is the dominant controlling factor in the rate of appearance of the drug in the blood, the focus on key API and formulation variables that affect the drug release and the corresponding in vitro test method is crucial in identification of a predictive test condition. Furthermore, continued pursuit of such a test not only can bring considerable benefit to product development and quality control, but it also will help advance scientific understanding and continued effort in developing new biopharmaceutical tools for improved prediction of in vivo drug absorption.

### 16.3.2 Drug and formulation considerations

The essential conditions for correlating in vitro dissolution with in vivo performance include (1) the apparent in vivo absorption is dissolution-rate limited; (2) in vitro drug release (dissolution and/or erosion) is the critical dosage form attributes; (3) test formulations exhibit different in vivo performances; (4) in vitro test is discriminating (IVIVR) and/or predictive (IVIVC) of in vivo performance. When these conditions are met, an IVIVC is deemed feasible. To develop an in vitro dissolution test that can be used to evaluate how changes in formulation and/or manufacturing processes may affect in vivo performance, integrated knowledge of drug properties, delivery technology, formulation, and biopharmaceutics is required to understand the specific challenges, choose the right biopharmaceutical tools, and design the appropriate studies.

#### *16.3.2.1 Immediate-release dosage forms*

It is generally accepted that an IVIVC is more difficult to achieve for IR dosage forms. With rapid drug release, the apparent drug absorption of the dosage form usually occurs in the upper small intestine where many factors other than dissolution are known to limit, affect, or contribute to the apparent absorption, as discussed previously. The short absorption phase, in most cases, is difficult to characterize, making Level A IVIVC modeling less likely than Level C or multiple Level C. Thus, the feasibility of an IVIVC is often drug and product dependent. For BCS class 2 drugs, of which dissolution is prolonged due to very low solubility and/or product and process characteristics, an

IVIVC is possible. One such example is the relatively slow-eroding polymer matrix of amorphous solid dispersions that contain a high level of carrier polymer(s) and are manufactured using a melt-extrusion process.[74,120] In the case of BCS class 1 drugs, an IVIVC is less likely unless the drug dissolution is significantly slowed due to formulation, processing, or the compound belongs to a borderline solubility classification. IVIVC is very rare for BCS class 3 drugs, of which gastric emptying, permeability, and transporters are typically involved in drug absorption. The opportunity for IVIVR or IVIVC may sometimes exist for BCS class 4 drugs, of which dissolution, permeability, and transporters can all influence the rate of in vivo absorption depending on the relative contributions of each factor and whether the low permeability classification is borderline or due to metabolism.[121] Challenges and various physicochemical, biopharmaceutical, and physiological factors that need to be considered in developing IVIVC and IVIVR of IR oral dosage forms have been reviewed extensively.[92,122] One of the major issues with assessing the feasibility of IVIVC using the BCS classification is that the boundaries for high solubility and permeability are set very conservatively.[92] As a result, each BCS class includes compounds with a broad range of properties and potentially different rate-limiting steps for absorption. For example, a low-solubility compound that narrowly misses the 90% fabs boundary would technically fall into BCS class 4, and yet it is very unlikely to have permeability-limited absorption. For such a compound, IVIVC remains possible. Similarly, certain BCS class 2 compounds are less likely to be candidates for developing an IVIVC.[92] Therefore, it is important to understand the specific characteristics of API and drug products, including the operating principles and manufacturing process. It should be noted that when a quantitative in vitro/in vivo relationship cannot be established or validated, an IVIVR that defines a dissolution range or space with demonstrated bioequivalence can also be useful in assuring acceptable in vivo performance via mapping in vitro and in vivo data of different formulations.[84,92,123]

#### 16.3.2.2 Extended-release (ER) dosage forms

Compared to IR products, an IVIVC is, in general, more desirable and readily defined for ER solid dosage forms. In fact, it is often expected that an in vitro release test is predictive or has an in vivo rank order because drug input from the GI tract is by design controlled or modified by the drug release from the dosage form. With an ER dosage form, the apparent absorption takes place in the small intestine, ascending colon, and/or throughout the large intestine depending on the API properties and the product design.[124] A longer absorption phase over an extended period of time, in principle, renders it possible to develop Level A, B, C or Multiple Level C IVIVC. However, matching in vivo drug release remains challenging due to significant heterogeneity in permeability and lumenal environments (eg, pH, water, surface area and contents, transporters, and gut wall metabolism) in different segments of the digestive tract. Thus, the feasibility of an IVIVC remains dependent on the drug molecule, delivery technology, formulation design of the dosage forms, and their interactions with an in vitro test method and condition. Investigation of IVIVC for ER dosage forms should start with understanding drug properties, doses, and their relationship with the associated formulation technologies discussed in chapter "Rational Design of Oral Modified-Release Drug Delivery Systems." In general, the in vivo apparent absorption of ER products containing soluble compounds (eg, BCS class 1) is primarily limited by the drug release from a diffusion or osmotically controlled ER system with fewer significant confounding factors. Thus, an IVIVC is more likely. With BCS class 2 drugs, solubility-to-dose ratio, drug-release mechanism, release duration, and absorption pathways can significantly influence the in vitro and in vivo relationship. In addition, drug particle dissolution following its release (metering) from the dosage form may also play a significant role depending on the dose and solubility of the drug substance. A detailed discussion is provided in an example in the case study section of this chapter. IVIVC for a typical BCS class 3 or 4 drug is more difficult to obtain because of the various factors often involved in its apparent absorption from the GI tract, including low and variable membrane permeability, transporters, and regional dependency.[75,76,78,79,125] In fact, many of the drugs in BCS class 3 or 4 are either not feasible for ER development or exhibit very limited absorption windows,[76,78] such as ranitidine, atenolol, furosemide, erythromycin, cefazolin, amoxicillin, hydrochlorothiazide, methotrexate, acyclovir, and neomycin.

Dosage form behavior as related to IVIVC development depends on drug property, delivery technology, and formulation design. During the drug release from ER systems across different segments of the GI tract, the drug and dosage form are subject to a wide range of environments and conditions, such as varying surface area, absorption pathways, permeability, metabolism, mixing, secretion, lumen content, and amount of fluids. As a result, the in vivo absorption from ER may significantly vary with regions, making its estimation for IVIVC modeling unreliable due to deviations from the system definition of Case III in Table 16.1. In addition, in vitro and in vivo mechanisms may differ or change depending on the drug and formulation design. Increased variability of the

**TABLE 16.5** Example of Extended-Release Systems and Food Effect on Bioavailability Parameters

| Product | Dosage form | $C_{max}$ | AUC | $T_{max}$ |
|---|---|---|---|---|
| Theophylline | IR tablet | Decrease (30–50%) | No change | Increase |
| Theo-Dur | Coated beads | Decrease (60%) | Decrease (50%) | Increase |
| Theo-24 | Coated beads | Increase (120%) | Increase (60%) | No change |
| Theo-Dur | Matrix tablet | No change | No change | No change |
| Uniphyl | Matrix tablet | Increase (70%) | Increase (70%) | Increase |
| Verapamil | IR tablet | Decrease (15%) | No change | Increase |
| Isoptin SR | Matrix tablet | Decrease (50%) | Decrease (50%) | Increase |
| Verelan PM | Coated beads | No change | No change | No change |
| Test Formulation 1[a] | Coated tablet | No change | No change | Increase |
| Test Formulation 2[a] | Coated beads | No change | No change | Increase |
| Covera HS | OROS tablet | No change | No change | No change |

[a]*Clin Pharmacol Ther, July, 77–83, 1985.*

apparent absorption often observed with ER products may also be exacerbated by improper or less robust formulation designs. A review of literature indicates a wide range of in vivo performance and/or relationships with in vitro drug release for many drugs formulated using different or the same types of delivery technologies. For example, part of the in vivo behavior of a solid product can be inferred from the effect of food on its PK characteristics, that is, food effect. Food intake is known to directly and indirectly induce changes in the GI environment and conditions, including gastric emptying, intestinal motility, mixing, mechanical and shear stress, lumen content, pH, viscosity, ionic strength, osmolality, secretions (bile salts and digestive enzymes), and the activity or capacity of metabolic enzymes and transporters.[27] There is no general in vitro or animal model that is predictive of the effect of such changes on drug absorption. However, the potential impact of these changes on the in vivo performance may be used as an indirect gauge for evaluation of the robustness of an ER solid product in IVIVC development. A survey of food effect on AUC and $C_{max}$ of 47 selected marketed ER products[126] shows that out of 32 soluble APIs, seven exhibit food effect on AUC and eight on $C_{max}$, while among 15 insoluble APIs, six exhibit food effect on AUC and 14 on $C_{max}$. Four out of seven osmotic pump products displayed food effect on AUC or $C_{max}$. Table 16.5 shows ER products of two well-known BCS class 1 drugs and their food effect.[127] It is evident that both ER technology and formulation play a significant role in how a product performs in the GI tract. Therefore, the opportunity and success of developing an IVIVC of ER dosage forms depends on individual APIs as well as the delivery systems and the formulation design.

**TABLE 16.6** Common Extended-Release Systems and IVIVC

| ER system | Characteristics |
|---|---|
| Matrix | • In vitro release sensitive to in vitro test conditions.<br>• In vivo results depend on individual drugs and formulation design.<br>• Hydrophilic matrix: Gel strength and system integrity also affect rate and mechanism of drug release in vivo.<br>• Possible to alter in vitro test condition for obtaining IVIVC. |
| Reservoir | • In vitro release typically sensitive to in vitro test conditions.<br>• In vivo results depend on individual drugs and formulation design.<br>• Possible to adjust test condition for obtaining IVIVC. |
| Osmotic pump | • In vitro release generally insensitive to test conditions.<br>• In vivo results depend on individual drugs.<br>• Higher probability to obtain IVIVC.<br>• Lack of flexibility to adjust test condition to match in vivo performance. |

Table 16.6 is a comparison of three broad types of ER drug delivery systems discussed in chapter "Rational Design of Oral Modified-Release Drug Delivery Systems" with respect to the characteristics related to IVIVC. Among the three types of ER technologies, it is well known that the drug release from an osmotic system is generally insensitive to in vitro test conditions, thus offering a higher chance of matching the in vivo drug release. However, once the in vitro test fails to predict, it is much more difficult to achieve IVIVC via altering the in vitro drug release of an osmotic system. On the other hand, the drug release from the other

two types of systems (reservoir and matrix) is most often dependent on the drug property and in vitro test methodology and conditions, thus providing greater flexibility and opportunity of adjusting the test variables to match the in vitro data with the in vivo profiles. It should be pointed out that for matrix systems, the drug release rate and mechanism are affected by formulation as well as system strength and integrity. The latter is also a function of formulation design. As a result, different formulations of the same drug molecule may exhibit different sensitivity to the test variables, which, in turn, can influence their respective in vitro/in vivo relationships. Therefore, it is important to evaluate key formulation attributes and variables that influence the behavior of a specific dosage form.

## 16.4 IVIVC DEVELOPMENT APPROACH

The scientific values and significant patient and operational benefits of exploring and establishing an IVIVC in the development of solid dosage forms have been well recognized.

It is important to create an IVIVC strategy and define a rational and effective approach for integration into the product development lifecycle in the project planning stage prior to initiating any development activities.

### 16.4.1 General strategy and approach

Exploration of IVIVC may start in the early, mid, or late stage of product development depending on the objective and resources of a development program. For example, an IVIVC can be investigated through review and evaluation of the historical in vitro and in vivo data or initiation of an IVIVC study at a later stage of development. This type of retrospective development strategy is usually either driven by the regulatory expectation or due to a lack of awareness of IVIVC and its value in the rational development of a solid product. To fully realize its potential, IVIVC development should begin at the early stage and continue throughout the formulation development cycle if necessary. A prospective or concurrent development strategy requires an integrated evaluation of a drug substance, ER technology, in vitro test method, and IVIVC feasibility followed by incorporating an IVIVC investigation in the formulation screening and development. When the IVIVC is explored as a part of the first in vivo formulation screening studies or subsequent development studies, it can be used to aid in the development of a project timeline, planning of formulation/process studies, and scale-up activities. If an IVIVC exists, it is advantageous to establish the model and validate it if necessary at the early stage to facilitate and accelerate subsequent product development, thereby saving time and resources associated with certain necessary bioequivalence studies. It also helps set the development and regulatory strategy. If an IVIVC is unlikely, a different product development strategy will have to be defined, for example, by planning bioequivalence studies required to support process scale-up and/or certain formulation changes in the development of an ER dosage form.

In the first stage of developing an ER product, two or more prototype formulations with different in vitro release rates are usually tested in humans in order to identify a formulation with a predefined PK performance (see chapter: Rational Design of Oral Modified-Release Drug Delivery Systems). With properly designed formulations and an in vitro test, the study can offer the first opportunity to explore and develop an IVIVC concurrently. For example, to enhance the chance of success or gain insight into an IVIVR, it is essential to consider building similar robustness in the prototype formulations to ensure a consistent release-control mechanism in vivo. This can be achieved via formulation design and additional testing prior to the in vivo test in humans. The former can be accomplished by considering key formulation variables that are likely to affect in vivo performance such as type and level of the rate-controlling materials, properties and loading of the drug and fillers, operating principle, and size and shape of the delivery system in designing the prototypes. The latter may involve challenging the prototypes through testing the in vitro behaviors (integrity, rank order of drug release) under different conditions (apparatus, medium, type and intensity of mixing, physicochemical and mechanical stress) or studying in an appropriate animal model. To fully exploit the information and potential of such in vivo studies for IVIVC, it is also beneficial to select and remain focused on one type of delivery technology in dosage form development. As discussed in chapter "Rational Design of Oral Modified-Release Drug Delivery Systems," when testing prototype formulations with drug-release profiles that bracket the theoretical target absorption profile in vivo, different outcomes may be obtained depending on the API, formulation design, and in vitro test methodology. Fig. 16.10 illustrates four examples of possible in vitro/in vivo relationships of such a study. Unless a quantitative IVIVC is obtained in the first study (Example I), further investigation is required to pursue an IVIVC depending on the outcome. Generally, if little difference is found in the in vivo performance, the in vitro test is considered overdiscriminating (Example IV). It would still be useful to modify the

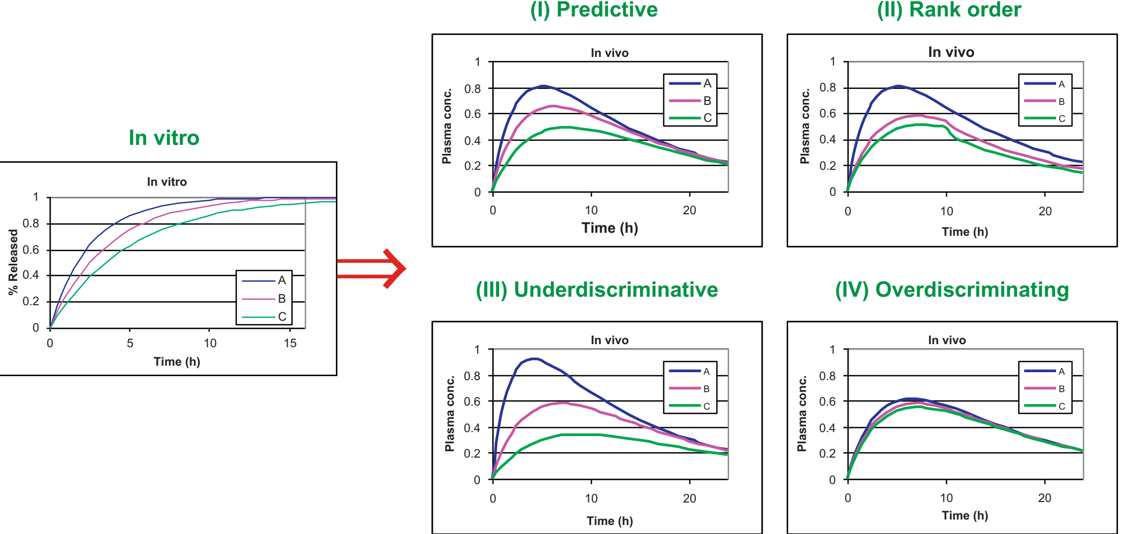



FIGURE 16.10 Examples illustrating possible outcome of the first bioavailability study of prototype ER formulations with varying in vitro release rates.

test conditions to achieve a similar in vitro performance for guiding formulation modifications in the subsequent in vivo studies. If the formulations show in vivo differences (rank order or underdiscriminating), the in vitro test conditions can be modified to correspond with the in vivo data for establishing an IVIVC. Adopting such an IVIVC strategy and making it a part of the dosage form development may take the following sequential steps:

1. Evaluate in vivo behaviors based on the understanding of drug's properties, dosage form, and their interplays with the GI tract.
2. Identify and investigate the mechanism/variables that likely influence or control both in vitro and apparent in vivo drug release.
3. Study test variables to identify condition(s) that:
   a. Differentiates between formulations with different in vivo behaviors.
   b. Deciphers the dosage form behavior and its relationship with the release-controlling variables (eg, critical formulation, processing parameters).
4. Design an in vitro test that matches in vivo performance (if feasible).
5. Establish, evaluate, and validate IVIVC.

It is evident that for formulations with varying PK profiles, developing an IVIVC relies heavily on the successful design of a discriminating in vitro test method.

## 16.4.2 Design of a predictive in vitro test

The first step in developing a predictive in vitro test is to ensure IVIVC feasibility, that is, that the drug release from the dosage form controls the drug input from the GI tract. While the in vitro data generated for an IVIVC are typically drug release, the estimated in vivo release or apparent absorption is the drug availability in vivo. As discussed previously, the latter is often a function of many other variables in addition to the drug release. Thus, the possibility of using an in vitro release test to predict the in vivo performance is drug and formulation dependent and should be explored on a case-by-case basis.

In the case of IR dosage form of which dissolution is limiting the rate of absorption (eg, certain BCS class 2 or 4 drugs), it is sometimes possible to develop dissolution tests for predicting differences in bioavailability among different formulations and dosing conditions. To achieve an a priori correlation, common approaches include altering the test apparatus or test conditions, some of which are described in Table 16.4. For example, to simulate the composition, volume, and hydrodynamics of the contents in the GI lumen, model compositions of the gastric and intestinal contents before and after meal intake were used as the dissolution media.[105] However, success as measured by meeting the rigorous IVIVC criteria for IR has been scarce, primarily because of the many confounding variables involved in apparent absorption in vivo.

In comparing in vivo with in vitro data of ER dosage forms, it is important to take into considerations a drug's properties, stability, and absorption pathways in relation to the effect of pH, transit time, location of drug release, food, ER technology utilized, and release-control mechanisms.[7] These types of integrated knowledge are essential in anticipating and evaluating the possible interplays of drug-GIT, formulation-GIT and

drug-formulation, and their impacts on the relationship between in vivo and in vivo data. While the results of an in vivo study are invariant, the evaluation of the API characteristics, delivery system, and formulation design can provide greater insight into the in vivo absorption behaviors to help assess IVIVC opportunity and develop a prognostic method.

In general, the observed difference between the in vivo apparent absorption or release mechanism and the in vitro data may be attributed to formulation factors (eg, robustness of the selected delivery technology or a particular composition), test method factors, and/or unique physicochemical and biopharmaceutical properties of the API (eg, low solubility, high dose, enterohepatic recycling, saturable metabolism, truncated absorption, effect of the absorbed drug or metabolite on the GI motility). Some of the drug-related factors can't be simulated by the in vitro test, but they can confound the deconvolution results or make the IR reference inappropriate as the UIR to estimate in vivo absorption if not taken into consideration.[33] For example, the in vivo absorption of the oxybutynin osmotic pump was found to be not only higher than that of the IR reference but also significantly longer (>24 hours) than the in vitro drug release (~15 hours) and the average GI residence time of solid dosage forms as well, resulting in a lack of IVIVC.[128] The former could be attributed to the reduced gut metabolism of the drug released in the lower bowel.[80] However, the latter observation was not understood because oxybutynin has neither known pharmacological effects on GI motility, such as opioid analgesics,[129] nor enterohepatic recycling. Wang et al. compared in vitro and in vivo data of a pulsatile release dosage form of methylphenidate (Ritalin LA). The double peaks were observed in plasma concentration-time profile results from two separate IR doses that were 4 hours apart.[130] This is likely a result of the favorable absorption properties combined with the low dose of methylphenidate. Slower absorption observed for the second phase can be attributed to the decreased in vivo dissolution rate of the second IR due to the changing drug-release environment in the lower bowel.

Sako et al.[131] investigated the effect of composition of a hydrophilic matrix on the in vivo release of acetaminophen. Three formulations containing different fillers showed similar in vitro dissolution profiles using different agitation intensity but had considerable different in vivo performances (Fig. 16.11). The observed discrepancy was believed to result from the different gel strengths of the matrix systems. In developing an in vitro test that differentiates the formulation behaviors, the test method was modified by subjecting the tablets to mechanical stress after 1 hour of dissolution (shaking with glass beads for 10 minutes), which resulted in drug-release profiles similar to those in vivo. It should be mentioned that in addition to creating artificial shear and attrition,[13,131] other techniques that are useful to characterize gel microstructure, formation, and strength of a hydrophilic matrix system include magnetic resonance imaging (MRI) microscopy, confocal microscopy, rheometry, texture analyzer, dynamic mechanical analyzer (DMA), and increasing the ionic strength in the dissolution medium.[132–138]

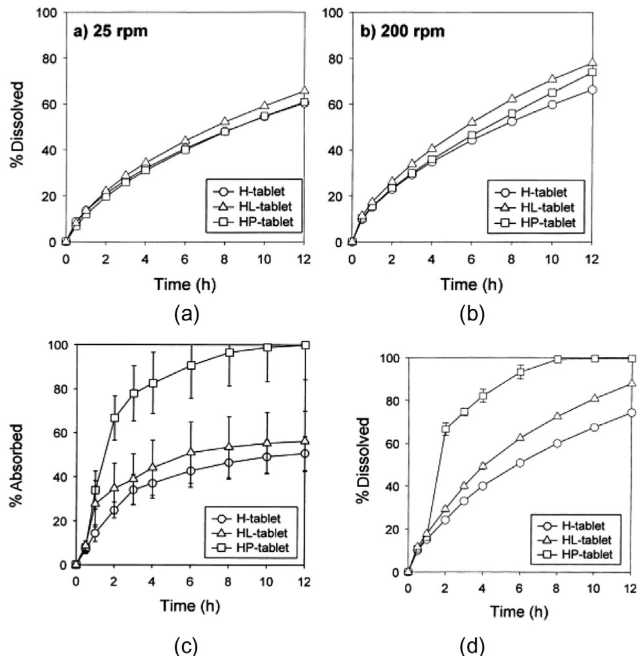



FIGURE 16.11 In vitro drug release and in vivo absorption profiles of three hydrophilic matrix formulations of acetaminophen: (a) and (b) conventional USP test; (c) in vivo absorption; (d) modified USP test. *Reprint with permission: J Control Release 2002;81(1–2):165–172.*

For high dose-to-solubility ratio drug substances that undergo drug dissolution upon delivery (metering) from the ER device, changing the regional dissolution and absorption environment (eg, surface area, amount of water, motility, enzymes, and transporters) may become a significant factor affecting the apparent in vivo absorption, thus IVIVC. For example, a discrepancy between the in vitro and in vivo data of the nifedipine osmotic pump was believed to be a result of the inability of conventional in vitro tests to separate release (metering) from dissolution due to large volume of test medium used throughout testing.[139] Generally, the in vitro test can become overdiscriminative or underdiscriminative of the in vivo results when particle dissolution upon release from an ER system plays a role in absorption, especially if a significant portion of the dose is delivered in the lower bowel.

An additional challenge in exploring a predictive in vitro test for an insoluble compound such as nifedipine is that its extent of absorption also depends on the particle size (or dissolution rate) of the drug upon its release from ER system,[140] which is difficult to reflect using a conventional test method. Furthermore, the potential influence of the solid phase of an insoluble drug substance on in vitro dissolution and in vivo absorption may also complicate the in vitro and in vivo relationship. For example, solid phase transition that occurs in situ or results from precipitation during drug release[141,142] may impact the in vitro and in vivo drug release in a different manner. It should be noted that these types of differences may affect the validity of using an IR reference as the UIR to estimate in vivo absorption.[33]

In investigating IVIVC for dosage forms with more complex release profiles or containing more than one drug substance (eg, bimodal, pulsatile release, and fixed-dose combination), it is essential to take into consideration both the properties of individual actives and their release characteristics. Depending on the release design feature, separate test methods may sometimes be required to match the in vivo absorption of the individual drugs or one part of the profile. It is also possible that a predictive test can only be developed for one of the actives or a portion of the dose and release profiles. For example, for a bimodal release profile consisting of an IR followed by an ER or an ER followed by an IR delivery (Fig. 16.12), it may be feasible to develop an IVIVC for only one (ER) or both portions of the curve. However, special attention is required when developing IVIVC models for more complex MR dosage forms. In a recent paper, Qiu et al. used three model drugs to illustrate the importance of understanding drug properties and product design in establishing and evaluating IVIVC of bimodal MR products.[143] Directly modeling in vitro and in vivo data without first considering drug properties, design characteristics, release mechanisms involved, and product behaviors may lead to a misleading, erroneous outcome or incorrect conclusions in the development of an IVIVC. For products containing more than one drug with synchronized or divergent-release profiles, various IVIVC outcomes are possible depending on a drug's BCS class, biological properties, dose, and IR-to-ER ratio. In any case, a predictive test method will always be useful whether it is for one or both drugs, a portion or the entire absorption profile in setting dissolution specifications and guiding or justifying changes to part of the formulation and process of interest.

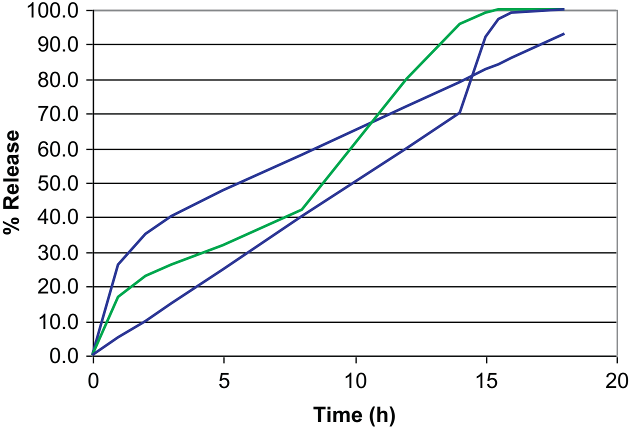



FIGURE 16.12 Illustration of bimodal drug release profiles.

In the method development process, the challenge frequently encountered is that the in vitro release not only differs from the in vivo release/absorption in rate and extent, but it may also exhibit a different release mechanism when a routine pharmacopoeial method is used (eg, USP II, 75 rpm, SIF). A general approach to developing an in vitro test for IVIVC requires identification of the mechanism that controls the in vivo drug release[7,144] For delivery systems that are sensitive to a release environment, the effects of test variables on release kinetics need to be investigated in order to understand how formulation variables and dosage form behaviors respond to environmental changes. In certain cases, formulations can be subject to more stressed conditions (eg, shear stress, high osmolality, ionic strength or agitation intensity, multiple apparatuses) to test robustness or to show difference. Subsequently, a qualitative or quantitative relationship between the drug release and key test variables may be established to guide the selection of a test condition that allows the in vitro data to match both the mechanism and the rates of in vivo release. To define this type of relationship, a well-planned experimental design based on understanding of the API, formulation, and their interplays with in vitro tests is more important than an attempt to simulate particular aspects of GI conditions without considering the specific issues associated with the formulation being studied. For example, switching to FaSSIF and/or FeSSIF media or a multicompartment dissolution system[118,145,146] is unlikely to resolve mismatch problems between in vitro and in vivo data related to a release mechanism change, gel strength, API-polymer interaction in vitro, impact of gut metabolism, or transporters or API on intestinal motility, as discussed previously.

Once an in vitro method that correlates with the in vivo absorption is established, its value as a quality control tool of a drug product is significantly enhanced. It serves as a tool to distinguish between acceptable and unacceptable drug products with respect to the in vivo performance. Lastly, it should be noted that a newly developed method cannot be

considered reliable and acceptable unless it has been validated and demonstrated to be predictive of different formulations with varying in vivo performance.

## 16.5 APPLICATIONS AND LIMITATIONS

The most important aspect of developing an IVIVC is to identify an in vitro test that can serve as an indicator of how a formulation will perform in vivo and how changes in formulation and/or processing may influence product performance. According to the FDA guidance on IVIVC for extended-release solid dosage forms,[2] a validated IVIVC can be used (1) for setting meaningful dissolution specifications to ensure product quality and in vivo performance and (2) for requesting a biowaiver so that the regulatory burdens, cost, and time associated with product development or postapproval changes can be significantly reduced.

### 16.5.1 Setting dissolution specifications

In vitro dissolution specifications are established to ensure batch-to-batch consistency and to differentiate between acceptable and unacceptable drug products, thus minimizing the possibility of releasing lots that might not have the desired in vivo performance. In general, dissolution behaviors of the clinical bioavailability batches are used to define the amount released at each time point.[1,2] Dissolution specification settings for IR products should be based on consideration of BCS classification and its ability discriminate with respect to changes in critical material, formulation, and process variables.[1,147] For ER products, challenges often arise in determining the acceptable variation around each time point for ER dosage forms. For new drug applications (NDAs) or abbreviated new drug applications (ANDAs), the specifications are based on the pivotal clinical batch or biobatch of a drug product. In the absence of an IVIVC, the range at any dissolution time point specification has to be within $\pm 10\%$ of the mean profile obtained from the biobatch. A deviation greater than 20% would be acceptable provided that the batches at the specification limits are bioequivalent.[1,2] For ER products, a minimum of three time points covering early, middle, and late stages of the profile are required, with dissolution of at least 80% at the last time point.

In setting dissolution specifications, a validated Level A IVIVC is most useful in ensuring that all lots within the specification limits are bioequivalent. In general, the convolution approach, as illustrated in Fig. 16.9, is preferred, and the specifications should be set on mean data using at least 12 dosage units. In determining the release limits, the dissolution curves defined by the upper and lower extremes established from the biobatch are convoluted to project the corresponding in vivo plasma concentration profiles. A maximum difference of 20% in the predicted $C_{max}$ and AUC is allowed between lots with the fastest and slowest release rates.[2,148] Alternatively, an acceptable set of plasma profiles representing formulations with faster and slower release rates relative to the biobatch can be used to set dissolution specifications by deconvolution based on the principles illustrated in Fig. 16.3. These curves selected based on extremes of 20% difference in $C_{max}$ and AUC are deconvoluted, and the resulting input curves are used to establish the upper and lower dissolution specification ranges at each time point via the IVIVC model.

In the case of Level C and Multiple Level C IVIVC, the specification ranges should be set at the correlation time point such that there is a maximum of 20% difference in the predicted AUC or $C_{max}$.[2] If the correlation involves more than one parameter, the one resulting in tighter limits should be used. In addition, drug release at the last time point should be at least 80%. Lake et al. reported an example of applying Level C IVIVC to set meaningful dissolution specifications using four carbamazepine IR tablets.[149] In cases where an IVIVC is absent or cannot pass validation criteria, it is still possible, though less optimally, to set a biorelevant specification using lots representing the upper and lower dissolution limits that have been shown to be bioequivalent to the clinical/bioavailability lots or to an appropriate reference standard. An example of establishing this type of biorelevant specification is provided in a case study of this chapter.

Although the general procedure and criteria for the establishment of dissolution specifications that ensure bioequivalence based on IVIVC have been proposed,[2,150,151] few discussions have centered on the detailed process and practical considerations in setting meaningful and realistic specification limits in product development. Elkoshi[152] described a procedure based on release rates that confines $C_{max}$ and AUC values within any desired range to set the minimum range specifications for both zero-order and first-order release products. In reviewing the methods for setting dissolution specifications, Hayes et al.[153] evaluated the most commonly adopted deterministic interpretation approach; that is, those batches passing the in vitro specifications would be bioequivalent, and those failing the specifications would not be bioequivalent if tested in vivo. According to the authors, the deterministic interpretation may not be appropriate, and the conditional probability needs to be considered due to random variation. Through a computer simulation based on an IVIVC model, the conditional

probabilities are shown to depend on the choice of dissolution specifications. The authors further described a method for optimizing the dissolution specifications that take production into consideration. A practical procedure of using IVIVC to establish dissolution specification based on scientific, regulatory, and operation considerations is provided in the case study section of this chapter.

### 16.5.2 Supporting waiver of in vivo bioavailability study

In addition to serving as a quality control test, comparative dissolution tests have been used to waive bioequivalence or bioavailability studies required for both IR and MR solid dosage forms under certain circumstances. Regulatory requirement including scientific basis, approaches, and evaluation criteria associated with these biowaivers have been clearly laid out in various regulatory guidelines. According to the regulatory guidelines,[1,154−156] the biowaiver request for investigational new drugs (INDs), investigational medicinal product dossiers (IMPDs), NDAs, MAs, ANDAs, and postapproval changes of IR solid dosage forms should be based on the consideration of a drug's BCS class,[157] therapeutic index, and potential effect of excipients on bioavailability. The global regulations with respect to biowaivers for IR solid oral products in the USA, the EU, Japan and from the World Health Organization (WHO) were reviewed by Guptaa et al.[6] To stimulate discussion of biowaivers and methods, the focus group on BCS and biowaivers of the International Pharmaceutical Federation (FIP) to date have published scientific data and biowaiver justifications for a total of 45 BCS class 1-3 compounds in the Journal of Pharmaceutical Sciences,[158] although many of them have not been accepted by regulatory agencies. These drug substances include: acetaminophen, acetazolamide, acetylsalicylic acid, acyclovir, amitriptyline hydrochloride, amodiaquine, hydrochloride, atenolol, bisoprolol fumarate, chloroquine phosphate, chloroquine sulfate, chloroquine hydrochloride, cimetidine, ciprofloxacin hydrochloride, codeine phosphate, diclofenac potassium, diclofenac sodium, doxycycline hyclate, efavirenz, ethambutol dihydrochloride, fluconazole, furosemide, ibuprofen, isoniazid, ketoprofen, lamivudine, levetiracetam, levofloxacin, mefloquine hydrochloride, metoclopramide hydrochloride, metronidazole, nifedipine, piroxicam, prednisolone, prednisone, primaquine diphosphate, propranolol hydrochloride, pyrazinamide, quinidine sulfate, quinine sulfate, ranitidine hydrochloride, ribavirin, rifampicin, stavudine, verapamil hydrochloride, and zidovudine (azidothymidine).

For modified-release products, a dissolution test based on a validated IVIVC can be used for obtaining a waiver for demonstrating in vivo bioavailability often required for NDAs, ANDAs, scale-up, and postapproval changes.[2,159] The criteria for granting the biowaivers using IVIVC are (1) the difference in predicted means of $C_{max}$ and AUC is no more than 20% from that of the reference product, (2) dissolution meets specifications. According to the FDA guidance, categories of biowaivers are also based on the therapeutic index of the drug, the extent of the validation performed on the developed IVIVC, and the dissolution characteristics of the formulation. For instance, for nonnarrow therapeutic index drugs, an IVIVC developed with two formulations can be used for a biowaiver in Level 3 manufacturing site changes and Level 3 nonrelease-controlling excipient changes defined in SUPAC Guidance for MR Solid Dosage Forms.[159] If an IVIVC is developed using three formulations, or two formulations with external validation, a biowaiver may include (1) Level 3 process changes, (2) complete removal or replacement of nonrelease-controlling excipients without affecting the release mechanism, (3) Level 3 changes in the release-controlling excipients, and (4) change of strength (lower than the highest strength).

### 16.5.3 Limitations and additional considerations

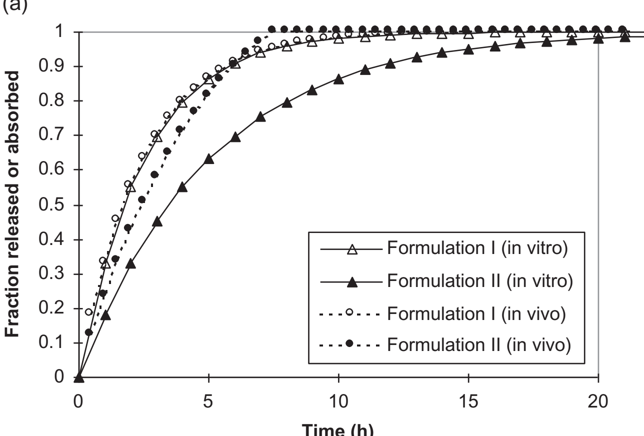
Limitations to the IVIVC methodology reside in the physicochemical, biological, and pharmacokinetic properties of the drug substance and the formulation design, as well as the methodology used to model, evaluate, and validate the IVIVC.

In the development of an IVIVC, the basic assumption of the linear system analysis is that the drug substance exhibits linear pharmacokinetic disposition. Thus, saturable absorption, absorption windows, rate-dependent absorption or rate-dependent presystemic metabolism, and enterohepatic recycling are important factors to consider when modeling and validating an IVIVC because they directly or indirectly result in deviation from the linear assumption.[12,160−163] In addition, an IVIVC should not be developed using plasma concentrations of racemate when there is stereoselective dissolution or absorption between the two enantiomers.[164] More importantly, the dissolution process should be the rate-limiting step in the absorption process, as discussed previously. In most cases, IVIVC models are being established using the average in vivo response, thus ignoring the intersubject and intrasubject variability. For drugs that have relatively high intersubject variability, it is important to take into account the intersubject and intrasubject variability in

constructing and evaluating the IVIVC model.[148,165,166] Cardot and Davit recently described some of the intricacies and possible traps related to the use of mean versus individual data, correction of formulations with different bioavailability, lag time and time scaling, the impact of intersubject and intrasubject variability, and the potential confounding effects of flip-flop kinetics. All of these factors must be considered to increase the chance of a successful IVIVC.[25] Lastly, the in vivo studies used for developing an IVIVC are conducted in healthy volunteers under a well-controlled environment. Factors that might affect the in vivo performance of the dosage form or physiology should also be considered,[167,168] such as food, disease state, age (pediatric and geriatric), and drug−drug or drug-GIT interactions, all of which can affect the GI motility and/or the GI transit time.

The state-of-the-art is such that an IVIVC is typically only valid for one particular type of dosage form containing rate-controlling excipients with the same release mechanism. Even with the same type of solid dosage form, such as a tablet, different release mechanisms (eg, diffusion vs osmosis) often necessitate the development of a separate IVIVC for the same drug molecule. In IVIVC modeling, the absorption parameters obtained with the most widely used Wagner−Nelson method reflect only the rate and not the extent of absorption. Problems can arise from a correlation established using formulations that have different systemic bioavailability. For example, decreased or truncated absorption in the lower GI tract may occur with slow-releasing formulations due to less liquid available for dissolution, lower permeability and surface area, the presence of bacterial metabolism, or a short residence time. As a result, the IVIVC will be apparently formulation-dependent if not corrected. This is illustrated using a simulated example. Two formulations (I and II) were originally designed to release a drug over approximately 8 and 14 hours (Fig. 16.13a). Following oral administration, decreased bioavailability of Formulation II was observed because the window of absorption is found to be approximately 8 hours (Fig. 16.13b). The apparent in vivo absorption profiles of the two formulations obtained by the Wagner−Nelson method are also shown in Fig. 16.13a. A comparison between the in vitro release and the in vivo absorption indicates a good 1:1 relationship for Formulation I and a significant deviation from the relationship for Formulation II as a result of overestimation of the in vivo absorption. Therefore, in developing an IVIVC, reduced AUC needs to be accounted for, for instance, by using time-dependent function: $X_{\text{in vivo}} = g(t)\ X_{\text{in vitro}}$ where $g(t)$ is a step function for truncated or site-dependent absorption.

(a)




(b)

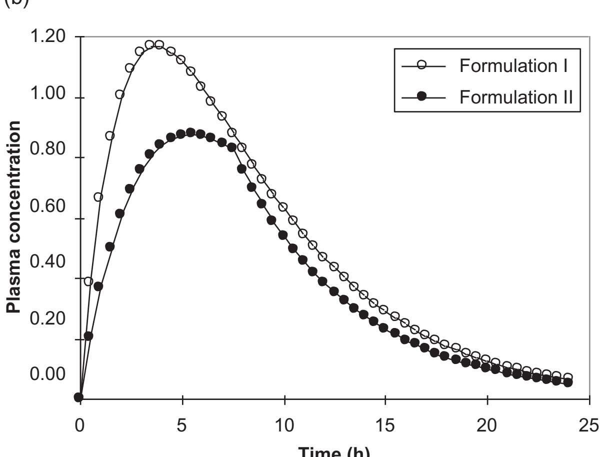



FIGURE 16.13 (a) In vitro release vs in vivo absorption profiles obtained by Wagner−Nelson method based on (b) Simulated plasma concentration profiles of two ER formulations with different release rates.

## 16.6 CASE STUDIES

### 16.6.1 Influence of API solubility on IVIVC

Nifedipine is practically insoluble. Its in vivo apparent absorption from the osmotic pump or matrix systems consists of sequential steps of release or metering of drug particles followed by particle dissolution and permeation across the intestinal membrane. This is indirectly supported by the known dependency of bioavailability on the drug particle size discussed previously. Conventional USP tests using a large volume of test medium containing a solubilizer to create sink conditions are incapable of separating the particle dissolution from the drug release. In investigating the IVIVC of Push-Pull osmotic pump of nifedipine, Grundy et al.[103] designed a two-phase test to measure the rate of drug transfer from an aqueous phase into an organic phase, that is, the processes of release of suspension from the device, particle dissolution, and

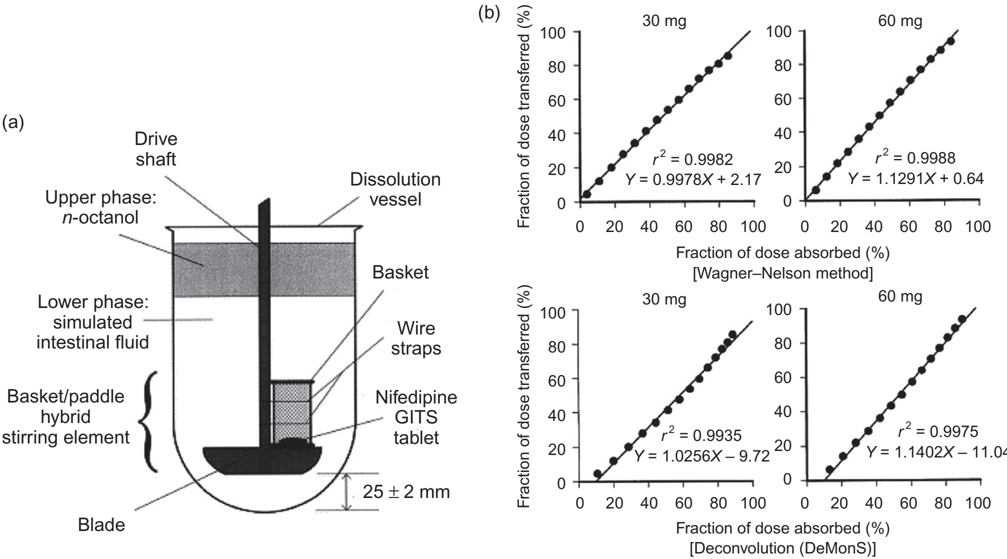



FIGURE 16.14 A Two-phase in vitro test system designed (a) for improving IVIVC of ER dosage form of an insoluble drug, nifedipine (b). *Reprint with permission: J Control Release 1997;48(1–8):9–17.*

subsequent partitioning into the organic phase (Fig. 16.14a). The authors demonstrated that a zero-order rate of drug transfer (0.96 mg/h) obtained from such a test system closely matched the estimated in vivo absorption rate of 1.03 mg/h (30-mg strength) as compared to a rate of 1.7 mg/h based on the conventional test. As a result, an improved 1:1 Level A IVIVC was obtained for all strengths ($R^2 > 0.99$). Similarly, the impact of the drug's solubility on IVIVR was also evaluated in studies comparing ER hydrophilic matrix formulations containing crystalline and amorphous compound with high dose-to-solubility ratio.[85,169] Three tablet formulations containing crystalline API and 10–30% hydroxypropyl methylcellulose (HPMC) exhibited different dissolution rates in a conventional USP method using 900 mL of test medium. However, the in vivo performance of the three formulations is similar, likely a result of a nonsink condition for the in vivo drug release. When the more soluble amorphous drug was used in the same type of ER matrices to improve the particle dissolution, a rank order relationship between the in vitro and in vivo data was observed in the same in vitro test.

## 16.6.2 Developing a predictive in vitro test[11,12,83]

Depakote ER tablet is a hydrophilic matrix-based, extended-release system with high drug loading. It provides approximately 20 hours of apparent zero-order in vivo absorption (Fig. 16.15). The active ingredient, divalproex sodium, is a stable and permeable compound with pH-dependent solubility. During early

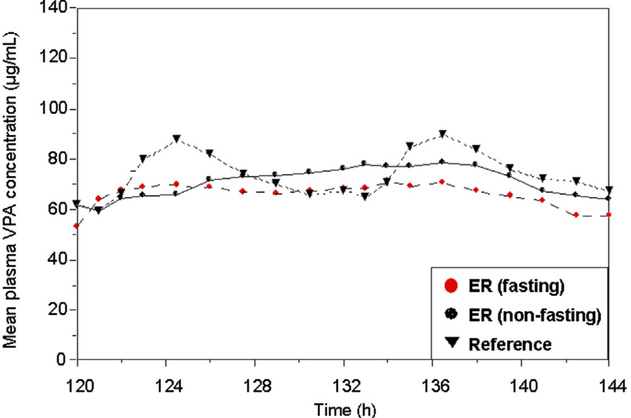



FIGURE 16.15 Mean steady-state plasma concentration profiles of once-daily Depakote ER tablet dosed under fasting and nonfasting conditions with twice-daily enteric Depakote tablet as reference.

formulation development, the in vitro drug release of three different formulations were all found to be slower and showed inadequate separation compared to in vivo absorption (underdiscriminating) when a conventional test was used (Fig. 16.16a). In addition, the mechanism of the in vitro release was diffusion controlled, whereas the apparent absorption profile obtained by deconvolution showed zero-order absorption, suggesting a predominantly erosion-controlled in vivo release (also supported by steady-state plasma concentration curves in Fig. 16.15).

In order to develop a new in vitro test that predicts in vivo absorption, statistically designed studies were carried out to investigate the effects of various in vitro testing variables on drug release. The variables

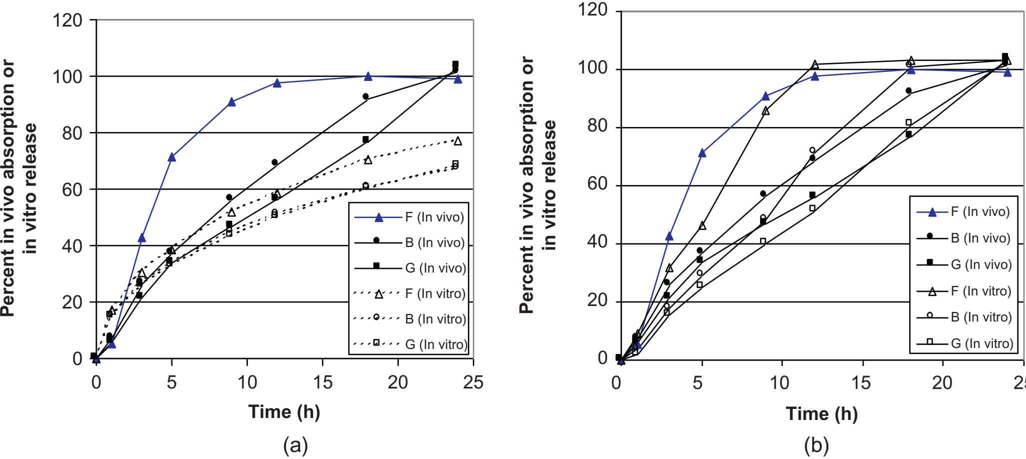



FIGURE 16.16 Mean in vivo absorption versus in vitro release profiles of three formulations using (a) conventional in vitro test method (USP II, 100 rpm, pH 7.5 phosphate buffer) and (b) a predictive test method (USP II, 100 rpm, 0.75 h 0.1 N HCl followed by 0.05 M phosphate buffer containing 75 mM SLS at pH 5.5).

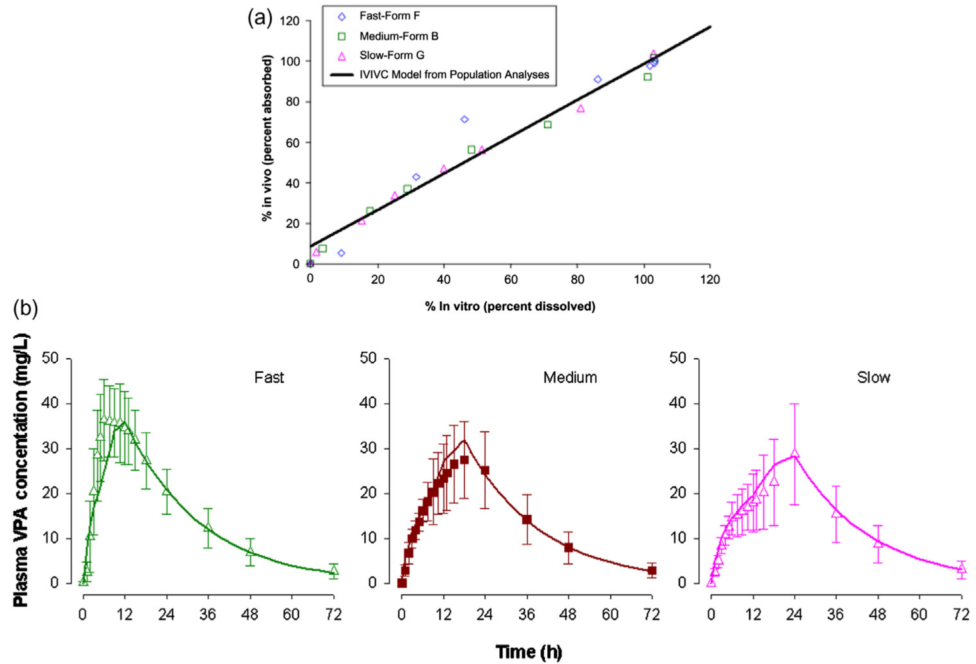



FIGURE 16.17 Results of in vivo/in vitro correlation studies of extended-release divalproex sodium tablets: (a) IVIVC plot and (b) mean predicted in vivo plasma profiles of three different formulations.

investigated included agitation intensity, apparatus, surfactant, pH, and ionic strength of the dissolution medium. Based on factorial studies and statistical analysis, a new set of test conditions was determined and demonstrated to correlate with the in vivo drug absorption for various ER formulations (Fig. 16.16b). Statistical evaluation of the in vitro method based on a hypothesis test indicated that the same IVIVC equation holds for the three different formulations (Fig. 16.17a). A mixed-effects model was used for data analysis in which the dependence among observations from the same subject in the human pharmacokinetic study was taken into account. Fig. 16.17b shows the agreement between the observed and the predicted plasma profiles of the tablets. The method has been successfully validated internally and externally on multiple occasions over a period of eight years. More importantly, it has been applied to (1) set meaningful drug-release specifications, (2) justify biowaivers for postapproval changes, and (3) assure product quality by timely

detecting significant dissolution changes during commercial production due to a change of the key rate-controlling polymer property that could not have been detected had the conventional dissolution test been used as a QC tool.[170]

This study illustrates a useful approach in identifying a predictive method for the development of an IVIVC, that is, adjusting the dissolution test conditions to correlate the in vitro data with the in vivo behaviors of the formulations.

### 16.6.3 Illustration of setting an optimal dissolution specification based on IVIVC using Monte Carlo simulation

The ability to establish a meaningful dissolution specification acceptable for commercial manufacturing has profound implications in ensuring consistent product performance and the routine production of the solid products. A specification based on product and process understanding assures both product quality and supply. If the specification range is unnecessarily narrow, the probability of failing a batch is increased due to the inherent variability of the raw materials, the product, and the manufacturing process. If a wide range is set for passing batches or solely based on the individual product and process capability, the in vivo performance may not always be ensured, especially in the absence of an IVIVC. One of the most significant advantages of establishing dissolution acceptance criteria using an IVIVC is that it offers greater flexibility for identifying a specification that maximize the probabilities of an assured product in vivo performance and a successful commercial production. More specifically, it allows for searching an optimal range in a multidimensional space defined by the needs of bioequivalence, quality control, manufacturability, and regulation.

One of major challenges in arriving at an optimal dissolution specification prior to regulatory filing is a lack of sufficient data to measure process capability at full production scale. To address this problem, Monte Carlo simulation can be used to evaluate acceptability of the proposed dissolution specifications with respect to manufacturability, bioequivalence, and regulatory requirement based on the inherent material, product, and process variability.[171–173] Monte Carlo simulation is a statistical tool for stochastic model calculations and analysis of propagation from uncertainties in model inputs into uncertainties in outputs (results).[174] It is commonly used to assess risk by making use of a pseudorandom drawing that simulates the real-life sampling to produce distributions of different outcome values. Its core idea is to use probability curves and random samples of inputs to explore the behavior of a complex stochastic system and determine/model the probability of different outcomes that cannot readily be predicted due to the intervention of random variables. Monte Carlo simulation generally includes four basic steps[175]: (1) define a domain of possible inputs, (2) generate inputs randomly from a probability distribution over the domain, (3) perform a deterministic computation on the inputs, and (4) aggregate the outputs for analysis. A number of statistical software, such as Minitab, Matlab, and MS Excel are often used to run Monte Carlo simulations.

This case study describes a useful approach to identify the dissolution specification limits that ensure product quality, performance, and robustness of commercial manufacturing. It is a data-driven approach that utilizes the available dissolution data, a validated IVIVC model, and Monte Carlo simulation, consisting of the following steps:

1. Based on the pivotal biobatch, generate multiple sets of specification ranges that meet bioequivalence criteria by confining differences in IVIVC model-predicted $C_{max}$ and AUC values within 20%.
2. Review and gather all existing dissolution data, which often include individual values of hundreds or thousands of individual tablets generated from a pilot scale, a larger scale, and stability studies during product development, and perform statistical analysis to estimate the probability distribution and variability of dissolution data resulting from natural variations in raw materials, product, processing, and test method.
3. Perform Monte Carlo simulation to select an acceptable specification that accommodates the inherent system variability. Specifically, dissolution data are generated using Monte Carlo simulation based on the statistical distribution (mean and spread) of the existing representative data. The simulated data are tested against the proposed specification limits using dissolution stage testing criteria (L1, L2, and L3). The simulation is typically iterated tens of thousands of times to estimate the overall probability of failing different stage testing associated with each set of proposed specification. For example, 100,000 production lots can be simulated by randomly sampling 100,000 groups of six tablets for dissolution stage testing when assessing individual sets of specification. The simulation is repeated for every set of proposed specifications until a set of specification is identified with minimum risk of failing the dissolution stage testing while ensuring all batches within the lower and upper specification limits are bioequivalent to one another.

Table 16.7 shows an illustrative example of this approach. Monte Carlo simulations were conducted

TABLE 16.7 Monte Carlo Simulation Results for Assessing Four Sets of Dissolution Specifications of an Extended-Release Tablet Based on IVIVC and Manufacturing Consideration

| Specification set | Q (2 h) | Q (8 h) | Q (12 h) | P[a] (Fail L3) | P (pass L1) | P (pass L2) | P (pass L3) |
|---|---|---|---|---|---|---|---|
| I | NML 20% | 35−66% | NLT 80% | 0.0% | 89.3% | 10.2% | 0.5% |
| II | NML 18% | 33−62% | NLT 80% | 0.0% | 78.2% | 12.3% | 0.5% |
| III | NML 18% | 36−60% | NLT 80% | 0.1% | 75.4% | 24.4% | 0.1% |
| IV | NML 20% | 38−58% | NLT 80% | 0.2% | 72.9% | 26.1% | 0.8% |

[a]*P: Probability of passing or failing dissolution stage testing.*

using four sets of specifications that meet the in vivo bioequivalence criteria for an ER tablet product. Sets I and II are considered acceptable for commercial manufacturing because they show a minimum risk of batch failures. It is worth noting that the accuracy and the reliability of this approach depend on the quality and the representativeness of the database used to estimate the inherent variability. Data from the commercial production following product approval should be collected and used to verify the simulation results and further improve the model if necessary.

## 16.6.4 Setting clinically relevant specifications

In science-based pharmaceutical development, IVIVC is recognized as one of the most important biopharmaceutical tools for enhancing drug product and process understanding and for ensuring consistent efficacy and safety throughout the drug product's lifecycle because it allows for the establishment of clinically relevant product specifications. Clinically relevant product specifications may be defined as those that can ensure the delivery of the intended dose at a consistent rate to patients to guarantee safety and efficacy profiles for the marked product relative to those achieved by the clinical trial formulations. By linking product quality to the clinical performance in product development, manufacturing, and continual improvement, clinically relevant specifications or controls associated with product critical quality attributes (CQA), critical material attributes (CMA), and critical process parameters (CPP) are expected to assure high product quality with a consistent safety and efficacy profile desired throughout lifecycle of a drug product. As a bridge between in vitro testing and in vivo exposure, an IVIVC can be effectively employed for understanding the impact of the drug product CQAs on in vivo performance. Through IVIVC, product quality specifications can be established optimally with assured clinical outcomes, using in vitro dissolution testing as a surrogate. For example, Duan et al. reported the use of IVIVC in setting the clinical relevant specifications for an ER matrix tablet.[176] During the development, three CMAs were identified as high-risk factors: particle size distribution (PS) of the API, viscosity of a matrix forming agent (MFA), and the PS of a matrix forming enhancing agent (MEA). Their impact on dissolution was investigated using a design of experiments (DOE). The data were analyzed by partial least square regressions and validated by comparing the model predicted and the observed dissolution values, along with a "leave one out" cross validation. The validated model was first used to predict dissolution at different values of MFA viscosity, particle sizes of MEA, and the API. The corresponding effects on AUC and $C_{max}$ were subsequently evaluated using a multiple Level C IVIVC. The predicted AUCs and $C_{max}$s were compared to those of the clinical batches with demonstrated efficacy and safety, and the ratios of AUC and $C_{max}$ were calculated and plotted against the three CMAs. For instance, Fig. 16.18 shows the combined effect of MFA viscosity and MEA PS on AUC ratio predicted using dissolution data at 3 hours under four different values of API particle size, expressed as the ratios of larger to smaller particle size (3.2, 6.6, 12.1 and 14.4). The rectangular box encloses the acceptable spaces for setting appropriate specifications, since they fall well within the bioequivalence range (0.8−1.25).

## 16.6.5 Setting biorelevant dissolution specification

Methylphenidate (MPH) is an amphetamine-like central nervous system stimulant commonly prescribed to treat attention-deficit/hyperactivity disorder (ADHD) in children, adolescents, and adults, as well as narcolepsy. It is a weak base with a $pK_a$ of 8.77 and a $\log P$ of 3.19. Its hydrochloride salt is freely soluble in water (18.6 mg/mL), stable, and well absorbed from the intestinal tract with a short elimination half-life of 3−4 hours.[177] These favorable properties combined with a low dose make MPH an ideal candidate for oral MR delivery. As a result, products using different MR

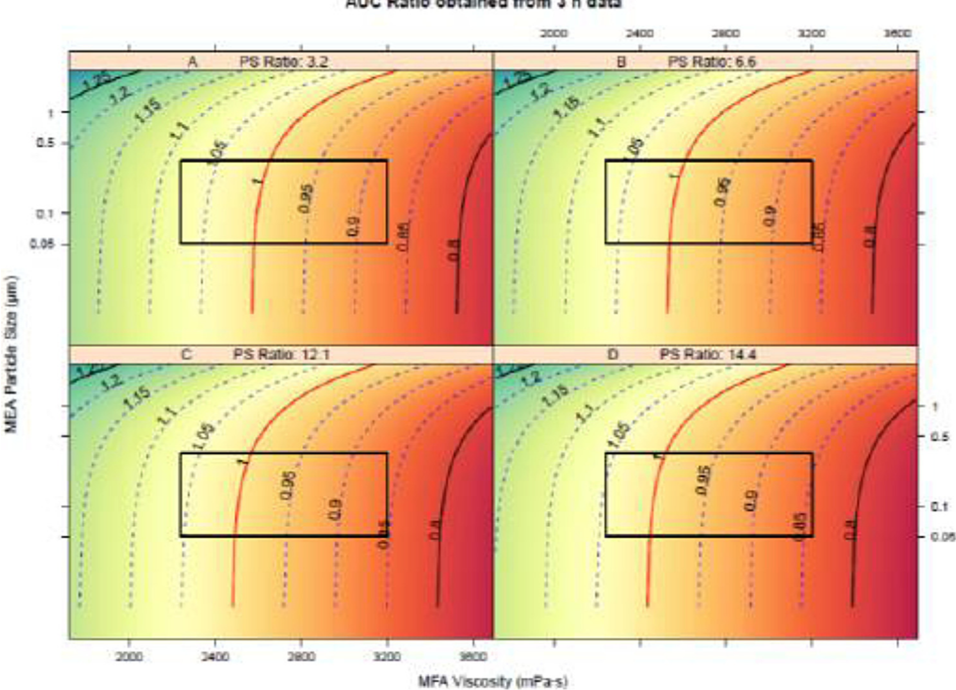



FIGURE 16.18 Effect of different combinations of three CMAs on AUC ratios predicted using the in vitro data at 3 h based on a Multiple Level C IVIVC.

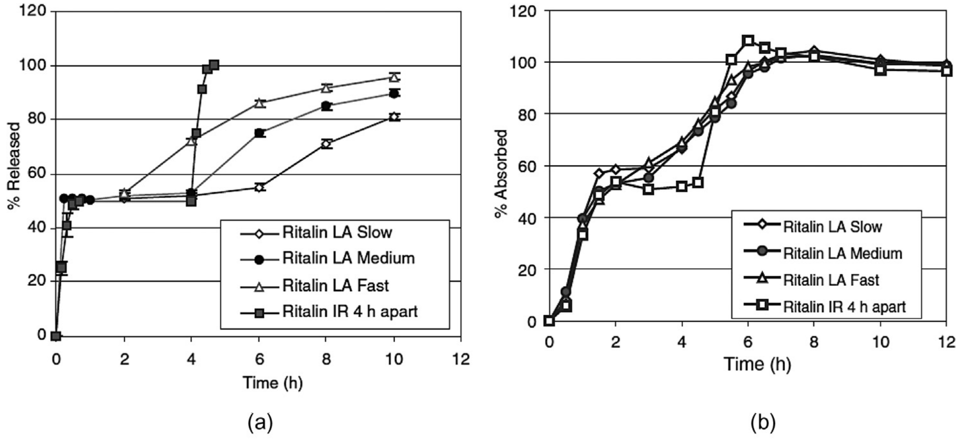



FIGURE 16.19 In vitro dissolution and in vivo absorption profiles of three Ritalin LA formulations and Ritalin IR tablet administered 4 hours apart.

design principles have been commercialized (see chapter: Product and Process Development of Solid Oral Dosage Forms). Wang et al. reported a study that evaluated the in vitro dissolution and in vivo absorption of MPH from a pulsatile release product (Ritalin LA capsule) using an IR formulation (Ritalin IR tablet) as a reference.[130] The Ritalin LA capsule consisting of 50% IR and 50% DR beads was designed to mimic PK performance of the IR product given 4 hours apart. In the study, three formulations, slow-, medium-, and fast-release, were prepared by varying the release rate of the DR portion by coating the IR beads to a different weight gain with an acrylates copolymer (Fig. 16.19a). The in vitro dissolution of the three MR formulations was determined using USP apparatus 1 (100 rpm) in 0.01N HCl for 2 hours followed by a pH 6.8 phosphate buffer. The three formulations were evaluated in 18 healthy volunteers under fasted conditions using a single-dose, randomized, four-way crossover design.

It was found that the three test formulations exhibited similar plasma concentration-time profiles with two peak concentrations and were bioequivalent

with respect to $C_{max}$ and AUC and the corresponding values describing the first and second peaks, although the in vitro dissolutions were different.[130] Fig. 16.19b showed that the absorption of MPH was biphasic, with a rapid absorption phase between 0 and ~2 hours and a slightly slower second absorption between ~3 and 6 hours. The results of this study can be used to justify and establish the biorelevant dissolution specification by defining a dissolution space that ensures bioequivalence in the absence of an IVIVC. More specifically, a wide range of dissolution specifications can be set based on the in vitro profiles of the fast and slow formulations, as shown in Fig. 16.19a, because batches prepared within the limits have been shown to be bioequivalent.

## 16.7 SUMMARY

The general concepts, theory, modeling methodology, assessment, and applications of in vitro/in vivo correlation have been established and extensively investigated, though differences in scientific approaches remain in the details of model development and evaluation. The state of the art is such that there is no universal in vitro model that can mimic or reproduce the highly complex and dynamic GI environment or predict the in vivo performance of solid oral dosage forms. Therefore, development of an IVIVC must be carried out case by case based on the understanding of the API properties, product characteristics, and their interplay with in vitro test method/conditions.

IVIVC is generally more likely for ER dosage forms than IR products, since drug absorption is typically limited by the drug release. To increase the chance of success, it is crucial to evaluate IVIVC feasibility of in vitro and in vivo results by integrating knowledge of physicochemical and biopharmaceutical characteristics of drug substances, dosage form design, and their interplays with the GI environment and in vitro test conditions. It is also important to make IVIVC strategy an integral part of the dosage form development program.

Once an IVIVC is developed and validated, the predictive in vitro test can be used as a surrogate for in vivo studies, a guide for setting meaningful product specifications, and a reliable tool for quality control. Whenever feasible, an IVIVC-based in vitro test method should be implemented in the QC laboratories such that any potential or unexpected changes of in vivo performance of a product during production can be detected to ensure safety and efficacy of every commercial batch.

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