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In Vitro – In Vivo Correlation (IVIVC): A Strategic Tool in Drug Development

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Abstract

In Vitro–In Vivo Correlation (IVIVC) plays a key role in pharmaceutical development of dosage forms. This tool hastens the drug development process and leads to improve the product quality. It is an integral part of the immediate release as well as modified release dosage forms development process. IVIVC is a tool used in quality control for scale up and post-approval changes e.g. to improve formulations or to change production processes & ultimately to reduce the number of human studies during development of new pharmaceuticals and also to support the biowaivers. This article provides the information on the various guidances, evaluation, validation, BCS application in IVIVC, levels of IVIVC, applications of IVIVC in mapping, novel drug delivery systems and prediction of IVIVC from the dissolution profile characteristics of product.

Keywords: IVIVC definitions; Predictions; BCS classification; IVIVC Levels; Applications, Guidance

Abbreviations: IVIVC: In Vitro In Vivo correlation; FDA: Food and Drug Administration; AUC: Area Under Curve; MDT vitro: Mean in vitro Dissolution Time; MRT: Mean Residence Time; BCS: Biopharmaceutical Classification System

Introduction

In vitro in vivo correlations (IVIVC) play a key role in the drug development and optimization of formulation which is certainly a time consuming and expensive process. Formulation optimization requires alteration in formulation, composition, equipments, batch sizes and manufacturing process. If such types of one or more changes are applied to the formulation, the in vivo bioequivalence studies in human may required to be done to prove the similarity of the new formulation which will not only increase the burden of carrying out a number of bioequivalence studies but eventually increase the cost of the optimization process and ultimately marketing of the new formulation. To overcome these problems it is desirable to develop in vitro tests that reflect can bioavailability data. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development. Thus, the main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers.

IVIVC is a mathematical relationship between in vitro properties of a dosage form with its in vivo performance. The In vitro release data of a dosage form containing the active substance serve as characteristic in vitro property, while the In vivo performance is generally represented by the time course of the plasma concentration of the active substance. These In vitro & In vivo data are then treated scientifically to determine correlations. For oral dosage forms, the in vitro release is usually measured and considered as dissolution rate. The relationship between the in vitro and in vivo characteristics can be expressed mathematically by a linear or nonlinear correlation. However, the plasma concentration cannot be directly correlated to the in vitro release rate; it has to be converted to the in vivo release or absorption data, either by pharmacokinetic compartment model analysis or by linear system analysis [1].

IVIVC definitions

United state pharmacopoeia (USP) definition of IVIVC

The establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form [2].

Food and drug administration (FDA) definition of IVIVC

An In-vitro in-vivo correlation (IVIVC) has been defined by the Food and Drug Administration (FDA) as "a predictive mathematical model describing the relationship between an in-vitro property of a dosage form and an in-vivo response".

Generally, the In vitro property is the rate or extent of drug dissolution or release while the In vivo response is the plasma drug concentration or amount of drug absorbed. Practically, the purpose of IVIVC is to use drug dissolution results from two or more products to predict similarity or dissimilarity of expected plasma drug concentration (profiles). Before one considers relating in vitro results to in vivo, one has to establish as to how one will establish similarity or dissimilarity of in vivo response i.e. plasma drug concentration profiles. The methodology of establishing similarity or dissimilarity of plasma drug concentrations profile is commonly known as bioequivalence testing. There are very well established guidances and standards available for establishing bioequivalence between drug profiles and products [3].

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Purpose of IVIVC

Reduction of regulatory burden

IVIVC can be used as substitute for additional in vivo experiments, under certain conditions.

Optimization of formulation

The optimization of formulations may require changes in the composition, manufacturing process, equipment, and batch sizes. In order to prove the validity of a new formulation, which is bioequivalent with a target formulation, a considerable amount of efforts is required to study bioequivalence (BE) /bioavailability (BA).

Justification for "therapeutic' product quality

IVIVC is often adequate for justification of therapeutically meaningful release specifications of the formulation.

Scale up post approval changes (Time and cost saving during the product development)

Validated IVIVC is also serves as justification for a biowaivers in filings of a Level 3 (or Type II in Europe) variation, either during scale-up or post approval, as well as for line extensions (e.g., different dosage strengths).

IVIVC as surrogate for *in vivo* bioequivalence and to support biowaivers (Time and cost saving)

The main purpose of an IVIVC model to utilize *in vitro* dissolution profiles as a surrogate for *in vivo* bioequivalence and to support biowaivers.

Levels of Ivivc

There are four levels of IVIVC that have been described in the FDA guidance, which include levels A, B, C, and multiple C [4]. The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form**.**

Level A correlation

An IVIVC that correlates the entire in vitro and in vivo profiles has regulatory relevance and is called a Level A Correlation **.**This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form [3,5].

Level A correlation is the most preferred to achieve; since it allows bio waiver for changes in manufacturing site, raw material suppliers, and minor changes in formulation. The purpose of Level A correlation is to define a direct relationship between in vivo data such that measurement of in vitro dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form.

Level B correlation

A level B IVIVC is based on the principles of statistical moment analysis. In this level of correlation, the mean in vitro dissolution time (MDT vitro) of the product is compared to either mean in vivo residence time (MRT) or the mean in vivo dissolution time (MDTvivo). MRT, MDTvitro and MDTvivo will be defined throughout the manuscript where appropriate [6]. A level B correlation does not uniquely reflect the actual in vivo plasma level curves, also in vitro data from such a correlation could not be used to justify the extremes of quality control standards hence it is least useful for regulatory purposes [5].

Level C correlation

Level C correlation relates one dissolution time point (t50%, t90%, etc.) to one mean pharmacokinetic parameter such as AUC, t_{max} or C_{max}. This is the weakest level of correlation as partial relationship between absorption and dissolution is established since it does not reflect the complete shape of plasma drug concentration time curve, which is the critical factor that defines the performance of a drug product.

Due to its obvious limitations, the usefulness of a Level C correlation is limited in predicting in vivo drug performance. In the early stages of formulation development Level C correlations can be useful when pilot formulations are being selected while waiver of an in vivo bioequivalance study (biowaiver) is generally not possible [5,6].

Multiple level C correlations

This level refers to the relationship between one or more pharmacokinetic parameters of interest (C_{max}, AUC, or any other suitable parameters) and amount of drug dissolved at several time point of dissolution profile. Multiple point level C correlation may be used to justify a biowaivers provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. 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. The development of a level A correlation is also likely, when multiple level C correlation is achieved at each time point at the same parameter such that the effect on the in vivo performance of any change in dissolution can be assessed [5,6].

Level D correlation

It is not a formal correlation but it is a semi quantitative (qualitative analysis) and rank order correlation and is not considered useful for regulatory purpose but can be serves as an aid in the development of a formulation or processing procedure [5,7] (Table 1).

IVIVC Models

The relationship of observed drug concentration-time profiles following administration of a tablet/capsule with drug dissolution and pharmacokinetics may be described graphically as shown in Figure 1.

It is generally assumed that absorption and dissolution have a

Level	In vitro	In vivo
A	Dissolution curve	Input (absorption) curves
B	Statistical moments: mean dissolution time (MDT)	Statistical moments: mean residence time (MRT), mean absorption time (MAT), etc
C	Disintegration time, time to have 10%, 50%, 90% dissolved, dissolution rate, dissolution efficiency (DE)	Maximum observed concentration (C_{max}), observed at time (T_{max}), absorption constant (Ka), Time to have 10, 50, 90% absorbed, AUC (total or cumulative)

A: one-to-one relationship between in vitro and in vivo data, e.g., in vitro dissolution vs. in vivo absorption

B: correlation based on statistical moments, e.g., in vitro MDT vs. in vivo MRT or MAT

C: point-to-point relationship between a dissolution and a pharmacokinetic parameter, e.g., in vitro T50% vs. in vivo T max, Multiple C: relationship between one or several PK parameters and amount dissolved at several time points.

Table 1: Various parameters used in IVIVC depending on the level.

linear relationship hence dissolution and absorption characteristics of a drug are commonly shown interchangeably. Thus from Figure 2, it is to be noted that one should be able to establish drug profiles with dissolution profiles combined with the pharmacokinetic characteristics of the drug as describe in the example above. This process of obtaining a drug profile from dissolution results is known as convolution. The opposite of this, i.e., obtaining or extracting a dissolution profile from a blood profile, is known as deconvolution Figure 2.

Convolution model

In the development of convolution model the drug concentration-time profiles obtained from dissolution results may be evaluated using criteria for *in vivo* bioavailability/ bioequivalence assessment, based on C_{max} and AUC parameters.

In mathematical terminology, dissolution results become an input function and plasma concentrations (e.g. from IV) become a weighting factor or function resulting in an output function representing plasma concentrations for the solid oral product.

Implementation of convolution-based method involves the production of a user-written subroutine for the NONMEM software package, has shown that a convolution-based method based on that of O'Hara et al. [9] produces superior results. Using the NONMEM package, a nonlinear mixed effects model can be fitted to the data with a time-scale model linking the *in vitro* and *in vivo* components [10].

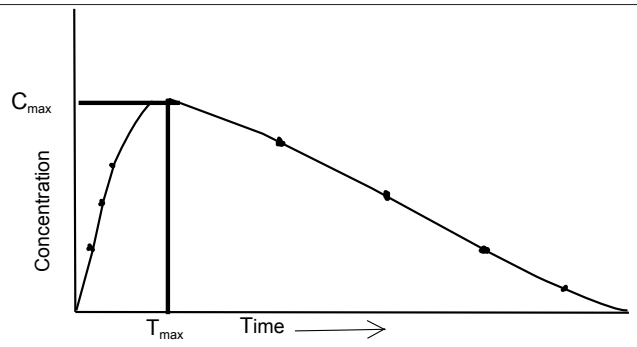

Figure 1: A typical drug concentration (in blood) – time profile reflecting the fate of a drug in the human body following an oral dose (tablet/capsule).

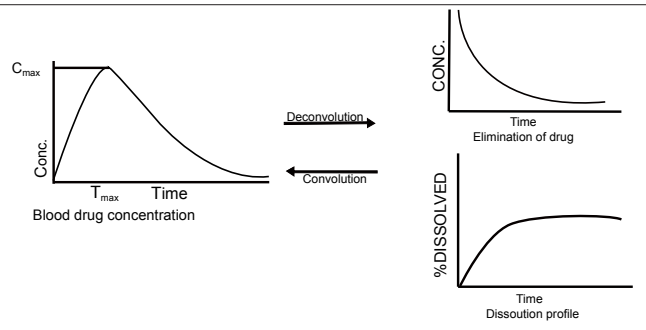

Figure 2: Schematic representation of deconvolution and convolution processes. Convolution is the process of combined effect of dissolution and elimination of drug in the body to reflect blood drug concentration-time profile (right to left). On the other hand, extracting dissolution profiles from blood drug concentration-time profile is known as the deconvolution process (left to right) [8].

It has been demonstrates that the convolution based and differential equation based models can be mathematically equivalent [11]. Software has been developed which implements a differential equation based approach. This method utilises existing NONMEM libraries and is an accurate method of modeling which is far more straightforward for users to implement. This research shows that, when the system being modeled is linear, the use of differential equations will produce results that are practically identical to those obtained from the convolution method.

But is a task that can be time consuming and complex [12]. As a result, this methodology, despite its advantages over the deconvolution-based approach, is not in widespread use.

Mathematically we can write the convolution as:

$$C(t) = C_{\delta}(t)F(t) = \int_0^t C_{\delta}(\tau)F(t-\tau)d\tau \quad (1)$$

Where, C(t) = Plasma drug concentrations after oral dose

Cδ(t) = Plasma concentrations after an IV dose or a dose of oral solution

Upon taking the derivative of C(t) with respect to time:

$$C(t) = C_{\delta}(t)F(t) + C_{\delta}(0)\int_0^t F(\tau)d\tau \quad (2)$$

When Cδ(0) = 0

$$C_{\delta}(t) * F(t) \quad (3)$$

Advantages of this approach relative to deconvolution-based IVIVC approaches include the following: The relationship between measured quantities (in vitro release and plasma drug concentrations) is modeled directly in a single stage rather than via an indirect two stage approach. The model directly predicts the plasma concentration time course. As a result the modeling focuses on the ability to predict measured quantities (not indirectly calculated quantities such as the cumulative amount absorbed). The results are more readily interpreted in terms of the effect of in vitro release on conventional bioequivalence metrics [5].

Deconvolution model

Deconvolution is a numerical method used to estimate the time course of drug input using a mathematical model based on the convolution integral.

The deconvolution technique requires the comparison of *in vivo* dissolution profile which can be obtained from the blood profiles with *in vitro* dissolution profiles. The observed fraction of the drug absorbed is estimated based on the Wagner-Nelson method. IV, IR or oral solution are attempted as the reference. Then, the pharmacokinetic parameters are estimated using a nonlinear regression tool or obtained from literatures reported previously. Based on the IVIVC model, the predicted fraction of the drug absorbed is calculated from the observed fraction of the drug dissolved. It is the most commonly cited and used method in the literature [10]. However this approach is conceptually difficult to use. For example: (1) Extracting *in vivo* dissolution data from a blood profile often requires elaborate mathematical and computing expertise. Fitting mathematical models are usually subjective in nature, and thus do not provide an unbiased approach in evaluating *in vivo* dissolution results/profiles. Even when *in vivo* dissolution curves are obtained there is no parameter available with associated statistical confidence and physiological relevance,

which would be used to establish the similarity or dissimilarity of the curves [13]. A more serious limitation of this approach is that it often requires multiple products having potentially different *in vivo* release characteristics (slow, medium, fast). These products are then used to define experimental conditions (medium, apparatus etc.) for an appropriate dissolution test to reflect their *in vivo* behavior. This approach is more suited for method/apparatus development as release characteristics of test products are to be known (slow, medium, fast) rather product evaluation [14].

Differential equation based approach

Another approach, has been proposed is based on systems of differential equations [15]. The use of a differential equation based model could also allow for the possibility of accurately modelling non-linear systems and further investigation is being carried out into the case where the drug is eliminated by a nonlinear, saturable process. The convolution and deconvolution methods assume that the system being modelled is linear but, in practice, this is not always the case. Work to date has shown that the convolution-based method is superior, but when presented with nonlinear data even this approach will fail. It is expected that, in the nonlinear case, the use of a differential equation based method would lead to more accurate predictions of plasma concentration.

The incorporation of time-scaling in the PDx-IVIVC equation allows this parameter to be estimated directly from the in vivoand vitro release data. As a result, the predictability of an IVIVC model can be evaluated over the entire in vivo time course.Internal predictability of the IVIVC model was assessed using convolution.PDx-IVIVC Model Equation:

$$x_{vivo}(t) = \begin{cases} 0, & t \geq 0. \\ a_1 + a_2 x_{vitro}(-b_1 + b_2 t), & t \geq 0. \end{cases} \quad (4)$$

For orally administered drugs, IVIVC is expected for highly permeable drugs, or drugs under dissolution rate-limiting conditions, which is supported by the Biopharmaceutical Classification System (BCS) [6,16]. For extended-release formulations following oral administration, modified BCS containing the three classes (high aqueous solubility, low aqueous solubility, and variable solubility) is proposed [17].

IVIVC Development

Any well designed and scientifically sound approach would be acceptable for establishment of an IVIVC. For the development and validation of a IVIVC model, two or three different formulations with different release rates, such as slow, medium, fast should be studied *In vitro* and *In vivo* [6].

A number of products with different release rates are usually manufactured by varying the primary rate controlling variable (e.g., the amount of excipient, or a property of the drug substance such as particle size) but within the same qualitative formulation. To develop a discriminative *in vitro* dissolution method, several method variables together with formulation variables are studied, e.g., different pH values, dissolution apparatuses and agitation speeds. Essentially at this stage a level A correlation is assumed and the formulation strategy is initiated with the objective of achieving the target in vitro profile. Development of a level A IVIVC model includes several steps.

In context of understanding the applications of IVIVR throughout the product development cycle, it is useful to become familiar with the following terms as they relate to a typical product development cycle for oral extended-release product [5].

An assumed IVIVC is the one that provides the initial guidance and direction for the early formulation development activity. Thus, during step 1 and with a particular desired product, appropriate in vitro targets are established to meet the desired in vivo profile specification. This assumed model can be the subject of revision as prototype formulations are developed and characterized in vivo, with the results often leading to a further cycle of prototype formulation and *In vivo* characterization.

Out of this product development cycle and *In vivo* characterization and, of course, extensive in vitro testing is often developed what can be referred to as retrospective IVIVC.

The defined formulation that meets the in vivo specification is employed for Stage 2. At this stage based on a greater understanding and appreciation of defined formulation and its characteristics, a prospective IVIVC is established through a well defined prospective IVIVC study [18,5].

Step 1

In the first step, the *In vivo* input profile of the drug from different formulations is calculated from drug concentrations in plasma (Figure 3). The target *In vivo* profile needs to be first established, based on, if possible, pharmacokinetic/pharmacodynamic models. Certainly, step 1 activity should culminate in a pilot PK study. This is typically a four or five-arm cross-over study. The size of this pilot pharmacokinetic study will vary depending on the inherent variability of the drug itself but typically range from 6 to 10 subjects [5]. The results of this pilot PK study provide the basis for establishing what has been referred to as a retrospective IVIVC .To separate drug input from drug distribution and elimination, model-dependent approaches, such as Wagner-Nelson and Loo-Riegelman, or model independent procedures, based on numerical deconvolution, may be utilised [19,20,21]. In step 1, the parameters that describe drug input rate, drug distribution and/or elimination are determined. In the model dependent approaches, the distribution and elimination rate constants describe pharmacokinetics after absorption. In the numerical deconvolution approach, the drug unit impulse response function describes distribution and elimination phases, respectively. The physicochemical characteristics of the drug substance itself, in relevance to formulation approach and dissolution at distal sites in the gastro-intestinal tract, need to be taken into account. Based on this information a priori in vitro methods are usually then developed and a theoretical in vitro target is established, which should achieve the desired absorption profile [5,18].

Step 2

By this phase of the development process, a defined formulation that meets the *In vivo* targets has been achieved. Extensive *In vitro* characterization is again performed across pH, media and apparatus, along with the consideration of results of stage 1. This leads to execution of a prospective IVIVC study. The IVIVC is developed and defined after an analysis of the result of that prospective in vivo study. It can often involved further in vitro method development in the context of the observed results, but clearly with the objective of establishing a definitive IVIVC. In this step, the relationship between

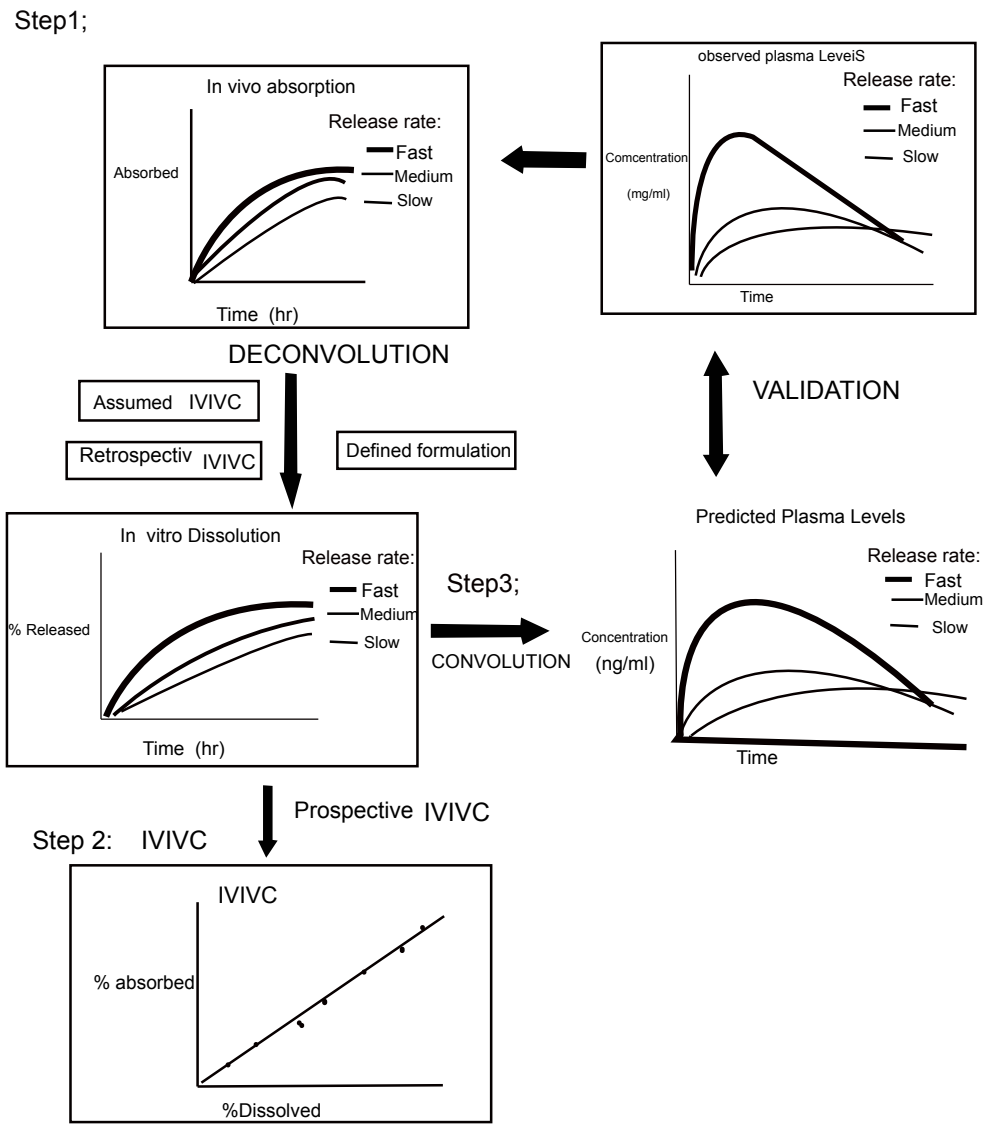

Figure 3: Development of IVIVC with validation.

in vitro dissolution and the *in vivo* drug input profile is determined (Figure 3). Either a linear or nonlinear relationship may be found. In some cases, time-scaling of *in vitro* data must be used, because *In vitro* dissolution and *In vivo* input may follow the same kinetics but still have different time-scales [6,22]. The time-scaling factor should be the same for all formulations if an IVIVC at level A is sought. During the early stages of correlation development, dissolution conditions may be altered to attempt to develop a 1-to-1 correlation between the in vitro dissolution profile and the *In vivo* dissolution profile. This work should also result in the definitive in vitro method that has been shown to be correlated with in vivo performance and sensitive to the specific formulation variables.

Step 3

In this phase plasma drug concentration profiles are predicted and compared to the observed time courses for different formulations (Figure 3). To generate predicted time courses, the drug input profile is predicted based on *In vitro* dissolution data and the *In vitro-In vivo* relationship generated in step 2. In the convolution process, the predicted drug input and parameters describing drug distribution and/or elimination phases are combined in order to get predicted time courses. This procedure, which includes steps 1-3, is called two-stage deconvolution. Alternatively, a drug input profile based on *in vitro* dissolution data can be solved together with parameters describing systemic pharmacokinetics, i.e. distribution and elimination. This approach is called direct convolution.

Different IVIVC model are used as a tool for formulation development and evaluation of immediate and extended release dosage forms for setting a dissolution specification and as a surrogate for bioequivalence testing.

As a result, considerable effort goes into their development and the main outcome is "the ability to predict, accurately and precisely, expected bioavailability characteristics for an extended release (ER) drug product from dissolution profile characteristics [10].

Once the IVIVC is established and defined it can be then used to guide the final cycle of formulation and process optimization program statistically based experimental design studies looking at critical formulation and process variables. This information can also be used into the activities of scale-up, pivotal batch manufacture, and process validation culminating in registration, approval and subsequent post-approval scale-up and other changes. Thus rather than viewing the IVIVC as a single exercise at a given point in a development program, one should view it as a parallel development in itself starting at the initial assumed level and being built on and modified through experience and leading ultimately to a prospective IVIVC".

Validation of IVIVC Model

Evaluation of predictability of IVIVC

Prediction errors are estimated for C_{max} and AUC to determine the validity of the correlation.

Various approaches of are used to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data.

Predictability of correlation

The objective of IVIVC evaluation is to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data. This objective should guide the choice and interpretation of evaluation methods. Any appropriate approach related to this objective may be used for evaluation of predictability [5,23]. It can be calculated by Prediction error that is the error in prediction of *in vivo* property from *in vitro* property of drug product (Figure 3).

Depending on the intended application of an IVIVC and the therapeutic index of the drug, evaluation of prediction error internally and/or externally may be appropriate [24].

Internal predictability

Evaluation of internal predictability is based on the initial data used to define the IVIVC model. Internal predictability is applied to IVIVC established using formulations with three or more release rates for non-narrow therapeutic index drugs exhibiting conclusive prediction error. If two formulations with different release rates are used to develop IVIVC, then the application of IVIVC would be limited to specified categories. The bioavailability (C_{max}, t_{max}/AUC) of formulation that is used in development of IVIVC is predicted from its *in vitro* property using IVIVC. Comparison between predicted bioavailability and observed bioavailability is done and % P.E is calculated. According to FDA guidelines, the average absolute %P.E should be below 10% and %P.E for individual formulation should be below 15% for establishment of IVIVC.

Under these circumstances, for complete evaluation and subsequent full application of the IVIVC, prediction of error externally is recommended [23].

Acceptance criteria

According to FDA guidance

1) ≤15% for absolute prediction error (%P.E.) of each formulation.

2) ≤ 10% for mean absolute prediction error (%P.E.).

External predictability

Most important when using an IVIVC as a surrogate for bioequivalence is confidence that the IVIVC can predict in vivo performance of subsequent lots of the drug product. Therefore, it may be important to establish the external predictability of the IVIVC.

Evaluation of external predictability is based on additional test data sets [5]. External predictability evaluation is not necessary unless the drug is a narrow therapeutic index, or only two release rates were used to develop the IVIVC, or, if the internal predictability criteria are not met i.e. prediction error internally is inconclusive [4,23]. The predicted bioavailability is compared with known bioavailability and % P.E is calculated. The prediction error for external validation should be below 10% whereas prediction error between 10-20% indicates inconclusive predictability and need of further study using additional data set [24].

The % prediction error can be calculated by the following equation:

Prediction error

For $\mathbf{C_{max}}$

$$\%\text{Prediction error (P.E.)}=\frac{(C_{max}\ observed - C_{max}\ predicted)}{C_{max}\ observed}\times 100 \quad (5)$$

For AUC:

$$\%\text{Prediction error (P.E.)}=\frac{(\text{AUC } observed - \text{AUC } predicted)}{\text{AUC } observed}\times 100 \quad (6)$$

Limitation of predictability metrics

Metrics used to evaluate the predictability is described simply the prediction error (%P.E.) for only two PK parameters i.e. C_{max} and AUC. E_{max} predicted with IVIVC model represents the maximum of the mean plasma profiles but is compared with the mean C_{max} observed calculated as the average of individual profile at different T_{max}. But T_{max} is not included in predictability metrics.

Factors to be Consider in Developing a Correlation

Biopharmaceutics classification system (BCS)

Biopharmaceutics Classification System (BCS) is a fundamental guideline for determining the conditions under which *in-vitro, in-vivo* correlations are expected [25]. It is also used as a tool for developing the *in-vitro* dissolution specification.

The classification is based on the drug dissolution and absorption model, which identifies the key parameters controlling drug absorption as a set of dimensionless numbers: the Absorption number, the Dissolution number and the Dose number [25-27].

The Absorption number is the ratio of the mean residence time to the absorption time.

$$A_n = T_{res} / T_{abs} + (\pi R^2 L / Q) / (R / P_{eff}) \quad (7)$$

The Dissolution number is a ratio of mean residence time to mean dissolution time given as equation 2

$$D_n = T_{res} / T_{diss} + (\pi R^2 L / Q) / (pr_0^2 / 3DC_s^{\min}) \quad (8)$$

The Dose number is the mass divided by an uptake volume of 250 ml and the drug's solubility as Equation 3

$$D_o = Dose / (V_o / C_s^{\min}) \quad (9)$$

The mean residence time here is the average of the residence time

in the stomach, small intestine and the colon. The fraction of dose absorbed then can be predicted based on these three parameters. For example, Absorption number 10 means that the permeation across the intestinal membrane is 10 times faster than the transit through the small intestine indicating 100% drug absorbed.

In the BCS, a drug is classified in one of four classes based solely on its solubility and intestinal permeability [27].

A biopharmaceutic drug classification scheme for correlating in vitro drug product dissolution and in vivo bioavailability is proposed based on recognizing that drug dissolution and gastrointestinal permeability are the fundamental parameters controlling rate and extent of drug absorption. This classification system was devised by Amidon et al. [27].

The drugs are divided into high/low-solubility and permeability classes. Currently, BCS guidelines are provided by USFDA, WHO, and EMEA (European Medicines Academy)

Class I: HIGH solubility / High permeability,

Class II: LOW solubility / High permeability,

Class III: HIGH solubility / LOW permeability,

Class IV: LOW solubility / LOW permeability.

Class I: High solubility- high permeability drugs

In case of class I , drugs (such as metoprolol) is well absorbed (though its systemic availability may be low due to first pass extraction/ metabolism) and the rate limiting step to drug absorption is drug dissolution or gastric emptying if dissolution is very rapid. The dissolution specification immediate release (IR) dosage forms of perhaps 85% dissolved in less than 15 min. May insure bioequivalence. To insure bioavailability for this case, the dissolution profile must be well defined and reproducible. [5,27].

Class II: Low solubility- high permeability drugs

This is the class of drugs (such as phenytoin) for which the dissolution profile must be most clearly defined and reproducible. More precisely this is the case where absorption number, (An) is high and Dissolution number (Dn) is low. Drug dissolution in vivo is then the rate controlling step in drug absorption and absorption is usually slower than for class I [28-31].

Class III: High solubility-low permeability drugs

For this class of drugs (such as cimetidine) Permeability is the rate controlling step in drug absorption. While the dissolution profile must be well defined, the simplification in dissolution specification as in Class I is applicable for immediate release dosage forms where drug input to the intestine is gastric emptying rate controlled.. Both rate and extent of drug absorption may be highly variable for this class of drugs, but id dissolution is fast i.e. 85% dissolved in less than 15 min, this variation will be due to the variable gastrointestinal transit, luminal contents , and membrane permeability rather than dosage form factors [5].

Class IV: Low solubility-low permeability drugs

This class of drugs present significant problems for effective oral delivery. The number of drugs that fall in this class will depend on the precise limits used from the permeability and solubility classification.

Applications

This concept underlying the BCS published finally led to introducing the possibility of waiving *in vivo* bioequivalence (BE) studies in favor of specific comparative *in vitro* testing to conclude BE of oral immediate release (IR) products with systemic actions [32].

In terms of BE, it is assumed that highly permeable, highly soluble drugs housed in rapidly dissolving drug products will be bioequivalent and that, unless major changes are made to the formulation, dissolution data can be used as a surrogate for pharmacokinetic data to demonstrate BE of two drug products. The BCS thus enables manufacturers to reduce the cost of approving scale-up and post approval changes to certain oral drug products without compromising public safety interests [33].

It is a drug-development tool that allows estimation of the contributions of three major factors, dissolution, solubility and intestinal permeability that affect oral drug absorption from IR solid oral dosage forms. It was first introduced into regulatory decision-making process in the guidance document on immediate release solid oral dosage forms: Scale-up and post approval changes [2]. BCS system is an indicator of developing a predictive IVIVC and also examined the importance of drug dissolution and permeability on IVIVC validity (Table 2).

The establishment of correlation needs, as described in the FDA or USP definitions, to use various parameters summarized in following table: Waiver of *in vivo* BE studies

Class	Solubility	Permeability	IVIVC correlation for IR Products
I	High	High	IVIVC correlation if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation
II	Low	High	IVIVC correlation expected if in in vitro dissolution rate is similar to in vivo dissolution rate , unless dose is very high
III	High	Low	Absorption [permeability] is rate determining and limited or no IVIV correlation with dissolution rate.
IV	Low	Low	Limited or no IVIV correlation expected.

Table 2: IVIV correlation expectation for immediate release product based on biopharmaceutic class.

Biopharmaceutics Drug Classification for Extended Release Drug Products **			
Class	**Solubility**	**Permeability**	**IVIVC**
IA	High & Site Independent	High & Site Independent	IVIVC Level A expected
IB	High & Site Independent	Dependent on site & Narrow Absorption Window	IVIVC Level C expected
IIa	Low & Site Independent	High & Site Independent	IVIVC Level A expected
IIb	Low & Site Independent	Dependent on site & Narrow Absorption Window	Little or no IVIVC
Va: Acidic	Variable	Variable	Little or no IVIVC
Vb: basic	Variable	Variable	IVIVC Level A expected.

Table 3: Biopharmaceutics Drug Classification for Extended Release Drug Products.

BCS Class	Examples	*Drug delivery technology*
Class I	Metoprolol, Diltizem, Verapamil, Propranolol, Acyclovir, Atropine, verapamil.	Macrocap, Micropump, MODAS (Multiporous oral drug absorption system), SCOT (Single composition osmotic tablet system), and SPDS (Stabilized pellet delivery system) [28,30,31].
Class II	Phenytoin, Danazole, Ketokonazole, Mefenamic acid, Tacrolimus, Piroxicam, griseofulvine, Warfarin,	Micronization, stabilization of high-energy states (including lyophilized fast-melt systems), use of surfactants, emulsion or microemulsion systems, solid dispersion and use of complexing agent such as cyclodextrins.e.g nanosuspension and nanocrystals are treated as hopeful means of increasing solubility and BA of poorly water-soluble active ingredients [28,30,31].
Class III	Cimetidine, Neomycin, ranitidine, Amoxycillin,	Oral vaccine system, Gastric retention system, High-Frequency Capsule and Telemetric Capsule [28,30,31].
Class IV	Cyclosporin A, Furosemide, Ritonavir, Saquinavir andTaxol.	The class IV drugs present a major challange for the development of drug delivery systems due to their poor solubility and permeability characteristics. These are administered by parenteral route with the formulation containing solubility enhancers [28,30,31].

Table 4: BCS class and drug delivery technology.

based on BCS Recommended for a solid oral Test product that exhibit rapid (85% in 30 min) and similar *in vitro* dissolution under specified conditions to an approved Reference product when the following conditions are satisfied (Table 3,4):

- Products are pharmaceutical equivalent
- Drug substance is highly soluble and highly permeable and is not considered have a narrow therapeutic range
- Excipients used are not likely to effect drug absorption

In vitro dissolution

Dissolution plays the important role in the formulation development as an obvious stage in IVIVC development when the dissolution is not influenced by factors such as pH, surfactants, osmotic pressure, mixing intensity, enzyme, ionic strength. Drug absorption from a solid dosage form following oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. The purpose of the *in-vitro* dissolution studies in the early stage of drug development is to select the optimum formulation, evaluate the active ingredient and excipients, and assess any minor changes for drug products. During the early stages of correlation development, dissolution conditions may be altered to attempt to develop a one-to-one correlation between the in vitro dissolution profile and the in vivo dissolution profile [5]. For the IVIVC perspective, dissolution is proposed to be a surrogate of drug bioavailability. Thus, dissolution standard may be necessary for the *in-vivo* waiver [26]. The dissolution methodology, which is able to discriminate between the study formulations and which best, reflects the *in vivo* behavior would be selected. Once a discriminating system is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation and should be fixed before further steps towards correlation evaluation are undertaken [34]. The types of dissolution apparatus used as per USP recommended in the FDA guidance especially, for modified release dosage form are specified by the USP and are:

[1] Rotating basket,

[2] Paddle method,

[3] Reciprocating cylinder,

[4] Flow through cell,

Other dissolution methodologies may be used, however, the first four are preferred, especially the basket and paddle. But primarily it is recommended to start with the basket or paddle method prior to using the others [26].

The *in vitro* dissolution release of a formulation can be modified to facilitate the correlation development. Changing dissolution testing conditions such as stirring speed, choice of apparatus, pH of the medium, and temperature may alter the dissolution profile.

As previously described, appropriate dissolution testing conditions should be selected so that the formulation behaves in the same manner as the *in vivo* dissolution.

For an appropriate dissolution test, in general and in particular for developing IVIVC, one requires to conduct the test selecting experimental conditions to simulate an *in vivo* environment as closely as possible. Commonly the following experimental conditions should be considered in this regard.

A common dissolution medium is dearated water, simulated gastric fluid (pH 1.2), or intestinal fluid (pH 6.8 or 7.4) without enzyme, and buffers with a pH range of 4.5 to 7.5 and be maintained at 37°C. For sparingly water-soluble drugs, use of surfactants in the dissolution medium is recommended [34,35]. A simple aqueous dissolution media is also recommended for BCS Class I drug as this type of drug exhibits lack of influence of dissolution medium properties [5,36]. Water and simulated gastric fluid then are the default mediums for most of the Class I drugs. A typical medium volume is 500 to 1000 ml.

1. Frequent samples (8-10) should be withdrawn to obtain a smooth dissolution profile leading to complete dissolution within the dosing interval of the test product in humans.
2. The normal test duration for immediate release is 15 to 60 minutes with a single time point. For example, BCS class I recommend 15 minutes. Additionally, two time points may be required for the BCS class II at 15 minutes and the other time at which 85% of the drug is dissolved [36].
3. In contrast, *in vitro* dissolution tests for a modified release dosage form require at least three time points to characterize the drug release. The first sampling time (1-2 hours or 20- 30% drug release) is chosen to check dose-dumping potential. The intermediate time point has to be around 50% drug release in order to define the *in vitro* release profile.
4. The dissolution medium should not be de-aerated. Preference should be given that the medium be equilibrated at 37°C with dissolved air/gasses, particularly for IVIVC studies.
5. An apparatus should be selected to have an appropriate

mechanism to provide thorough but gentle mixing and stirring for an efficient product/medium interaction. Use of sinkers may be avoided as these often alter the dissolution characteristics of the test products. Paddle and basket apparatuses are known for their inefficient stirring and mixing, thus their use should be critically evaluated before use for IVIVC studies.

6. The last time point is to define essentially complete drug release. The dissolution limit should be at least 80% drug release. Further justification as well as 24 hours test duration are required if the percent drug release is less than 80 [34,37].

7. If the dissolution results are not as expected, then the product/formulation should be modified to obtain the desired/expected release characteristics of the product. However, altering experimental conditions such as medium, apparatus, rpm etc. should be avoided as these are generally linked to GI physiology which remains the same for test to test or product to product. Obtaining dissolution results by altering testing (experimental) conditions may void the test for IVIVC purposes.

Once the discriminatory system is established, dissolution testing conditions should be fixed for all formulations tested for development of the correlation [6]. A dissolution profile of percentage or fraction of drug dissolved versus time then can be determined.

Comparison between dissolution profiles could be achieved using a difference factor (f_1) and a similarity factor (f_2) which originates from simple model independent approach. The difference factor calculates the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves:

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] / \left[\sum_{t=1}^{n} |R_t| \right] * 100 \right\} \quad (10)$$

Where, n is the number of time points, Rt is the dissolution value of the reference batch at time t, and Tt is the dissolution value of the test batch at time t. The similarity factor is a logarithmic reciprocal square root transformation of the sum squared error and is a measurement of the similarity in the percent dissolution between the two curves

$$f_2 = 50 * \log \left\{ \left[1 + (1/n) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} * 100 \right\} \quad (11)$$

Generally, f1 values up to 15 (0-15) and f2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves. The mean in vitro dissolution time (MDTvitro) is the mean time for the drug to dissolve under in vitro dissolution conditions. This is calculated using the equation 6:

$$MDT_{vitro} = \int_0^{\infty} (M_{\infty} - M(t))dt / M_{\infty} \quad (12)$$

For the IVIVC development, the dissolution profiles of at least 12 individual dosage units from each lot should be determined. The coefficient of variation (CV) for mean dissolution profiles of a single batch should be less than 10%. Since dissolution apparatuses tend to become less discriminative when operated at faster speeds, lower stirring speeds should be evaluated and an appropriate speed chosen in accordance with the test data. Using the basket method the common agitation is 50-100 rpm; with the paddle method, it is 50-75 rpm and 25 rpm for suspension [5].

In vivo absorption (Bioavailability studies)

The FDA requires *in vivo* bioavailability studies to be conducted for a New Drug Application (NDA). A bioavailability study should be performed to characterize the plasma concentration versus time profile for each of the formulation. These studies for the development of IVIVC should be performed in young healthy male adult volunteers under some restrictive conditions such as fasting, non-smoking, and no intake of other medications. In prior acceptable data sets, the number of subjects has ranged from 6 to 36. Although crossover studies are preferred, parallel studies or cross-study analyses may be acceptable. The latter may involve normalization with a common reference treatment. The drug is usually given in a crossover fashion with a washout period of at least five half-lives.

The bioavailability study can be assessed via plasma or urine data using the following parameters: (I) area under the plasma time curve (AUC), or the cumulative amount of drug excreted in urine (Du), (II) maximum concentration (C_{max}), or rate of drug excretion in urine (dDu/dt), and (III) a time of maximum concentration (T_{max}).

Several approaches can be used for determining the *In vivo* absorption. Wagner-Nelson, Loo-Riegelman, and numerical deconvolution are such methods [2,37]. Wagner Nelson and Loo-Riegelman are both model dependent methods in which the former is used for a one-compartment model and the latter is for multi-compartment system.

The Wagner Nelson method is less complicated than the Loo-Riegelman as there is no requirement for intravenous data. However, misinterpretation on the terminal phase of the plasma profile may be possible in the occurrence of a flip flop phenomenon in which the rate of absorption is slower than the rate of elimination.

Application of An IVIVC

Application in drug delivery system

Various rate controlling technologies are used as the basis for Modified release dosage forms e.g. Diffusion-dissolution, matrix retardation, osmosis, etc. to control, and prolong the release of drugs, for the administration by oral or parenteral route [24,38].

The novel drug delivery systems have been developed such as OROS, liposomes, niosomes, pharmacosomes, microspheres, nanoparticles, implants, in situ gelling system, organogels, transdermal drug delivery systems, parenteral depots, etc. as a substitute for conventional dosage forms. The obvious objective of these dosage forms is to achieve zero-order, long term, pulsatile, or "on demand" delivery. Major applications of IVIVC related to oral drug delivery and a few issues related to the development of IVIVC models for parenteral drug delivery are addressed herewith [39].

In early stages of drug delivery technology development

The most crucial stage in the drug development is drug candidate selection. Such selection is primarily based on the drug "developability" criteria, which include physicochemical properties of the drug and the results obtained from preformulation, preliminary studies involving several *in vitro* systems and *in vivo* animal models, which address efficacy and toxicity issues [24,40]. During this stage, IVIVC (exploring the relationship between in vitro and in vivo properties) of the drug in animal models provide an idea about the feasibility of the drug delivery system for a given drug candidates. In such correlations, study designs including study of more than one formulation of the modified-release

dosage forms and a rank order of release (fast/slow) of the formulations should be incorporated. Even though the formulations and methods used at this stage are not optimal, they promise better design and development efforts in the future.

Formulation assessment: In vitro dissolution

A suitable dissolution method that is capable of distinguishing the performance of formulations with different release rates in vitro and in vivo is an important tool in product development. Depending on the nature of the correlation, further changes to the dissolution method can be made. When the discriminatory in vitro method is validated, further formulation development can be relied on the in vitro dissolution only.

Dissolution specifications

Modified-release dosage forms typically require dissolution testing over multiple time points, and IVIVC plays an important role in setting these specifications [24,39]. Specification time points are usually chosen in the early, middle, and late stages of the dissolution profiles. In the absence of an IVIVC, the range of the dissolution specification rarely exceeds 10% of the dissolution of the pivotal clinical batch. However, in the presence of IVIVC, wider specifications may be applicable based on the predicted concentration-time profiles of test batches being bioequivalent to the reference batch.

The process of setting dissolution specifications in the presence of an IVIVC starts by obtaining the reference (pivotal clinical batch) dissolution profile. The dissolution of batches with different dissolution properties (slowest and fastest batches included) should be used along with the IVIVC model, and prediction of the concentration time profiles should be made using an appropriate convolution method. Specifications should optimally be established such that all batches with dissolution profiles between the fastest and slowest batches are bioequivalent and less optimally bioequivalent to the reference batch. The above exercise in achieving the widest possible dissolution specification allows majority of batches to pass and is possible only if a valid Level A model is available [24].

Future biowaivers

Frequently, drug development requires changes in formulations due to a variety of reasons, such as unexpected problems in stability, development, availability of better materials, better processing results, etc. Having an established IVIVC can help avoid bioequivalence studies by using the dissolution profile from the changed formulation, and subsequently predicting the in vivo concentration-time profile [24,41].

This predicted profile could act as a surrogate of the in vivo bioequivalence study. This has enormous cost-saving benefit in the form of reduced drug development spending and speedy implementation of post-approval changes. The nature of post-approval changes could range from minor (such as a change in non release-controlling excipient) to major (such as site change, equipment change, or change in method of manufacture, etc) [24,42].

IVIVC - Parenteral drug delivery

IVIVC can be developed and applied to parenteral dosage forms, such as controlled-release particulate systems, depot system, implants, etc, that are either injected or implanted. However, there are relatively fewer successes in the development of IVIVC for such dosage forms, which could be due to several reasons, a few of which are discussed further. Sophisticated modeling techniques are needed to correlate the *in vitro* and *in vivo* data, in case of burst release which is unpredictable and unavoidable [24,43].

Potent Drugs & Chronic Therapy - In general, several parenteral drug delivery systems are developed for potent drugs (eg, hormones, growth factors, antibiotics, etc) and for long-term delivery (anywhere from a day to a few weeks to months). In such instances, to establish a good IVIVC model, the drug concentrations should be monitored in the tissue fluids at the site of administration by techniques such as microdialysis, and then the correlation should be established to the *in vitro* release.

Biowaivers

Validated IVIVC is applicable to serve as justification for a biowaiver in filings of a Level 3 (or Type II in Europe) variation, either during scale-up or post approval, as well as for line extensions (e.g., different dosage strengths). A biowaiver will only be granted if the prediction of the in vivo performance of the product with the modified in vitro release rate remains bioequivalent with the originally tested product (i.e., the new dissolution rate remains within the IVIVC based biorelevant corridor).

The FDA guidance outlines five categories of biowaivers: 1) biowaivers without an IVIVC, 2) biowaivers using an IVIVC: non-narrow therapeutic index drugs, 3) biowaivers using an

IVIVC: narrow therapeutic index drugs, 4) biowaivers when in vitro dissolution is independent of dissolution test conditions and 5) situations for which an IVIVC is not recommended for biowaivers Biowaivers may be granted for manufacturing site changes, equipment changes, manufacturing process changes, and formulation composition changes according to a predictive and reliable IVIVC. The changes may range from minor changes that are not significant to alter product performance to major ones where an IVIVC is not sufficient to justify the change for regulatory decision [4,24].

Establishment of dissolution specifications

It is relatively easy to establish a multipoint dissolution specification for modified-release dosage forms .The dissolution behavior of the biobatch maybe used to define the amount to be released at each time point. However, the difficulty arises in the variation to be allowed around each time point [37]. The FDA guidance describes the procedures of setting dissolution specifications in cases of level A, multiple levels C, and level C correlation and where there is no IVIV correlation. Once an IVIVC developed, IVIVC should be used to set specifications in such a way that the fastest and lowest release rates allowed by the upper and lower dissolution specifications result in a maximum difference of 20% in the predicted C_{max} and AUC. Predicted plasma concentration and consequent AUC and C_{max} could be calculated using convolution or any other appropriate modeling techniques [24]. In the case of multiple level C correlation, the last time point should be the time point where at least 80% of drug has dissolved. For level C correlation, reasonable deviations from ±10 % may be acceptable if the range at any time point does not exceed 25%. When there is no IVIVC, the tolerance limits may be derived from the spread of in vitro dissolution data of batches with demonstrated acceptable in vivo performance (biobatch) or by demonstrating bioequivalence between batches at the proposed upper and lower limit of the dissolution range (the so called side batch

concept). Variability in release at each time point *is* recommended not to exceed a total numerical difference of ±10% (a total of 20%) or less of the labeled claim. In certain cases, deviations from this criterion can be acceptable up to a maximum range of 25%. Beyond this range, the specification should be supported by bioequivalence studies [43].

Mapping

Mapping is a process which relates Critical Manufacturing Variables (CMV), including formulation, processes, and equipment variables that can significantly affect drug release from the product. The mapping process defines boundaries of in vitro dissolution profiles on the basis of acceptable bioequivalency criteria. The optimum goal is to develop product specifications that will ensure bioequivalence of future batches prepared within the limits of acceptable dissolution specifications. Dissolution specifications based on mapping would increase the credibility of dissolution as a bioequivalency surrogate marker and will provide continuous assurance and predictability of the product performance [5].

Some Limitations in the IVIVC Arising from the In Vivo Data

Could easily be understood:

1. More than one dosage form is needed and if possible intravenous or solution is essential to calculate deconvolution.

2. Pharmacokinetics and absorption of the drug should be "linear." If the pharmacokinetic processes are dependent on the fraction of dose reaching the systemic blood flow (or of the dose administered) or on the rate of absorption, comparison between formulation and simulation cannot be made. This non-linearity may be owing to saturable absorption processes (active absorption), induction or inhibition of the metabolism, the first past effect, which is rate/absorption dependent, etc. Those points must be studied before any attempt to establish an IVIVC.

3. Absorption should not be the limiting factor, if the solubility is not the limiting factor in comparison to the drug release, an IVIVC may be attempted. The release must depend on the formulation, and must be the slowest phenomenon vs. dissolution and absorption.

Conclusions

The pharmaceutical industry has been striving to find a ways to saving precious resources in relevance to the budgets and increasing cost of drug development. IVIVC is a tool applied in various areas and stages of drug development to find a place in the regulatory bodies around the world. IVIVC can serve as surrogate for in vivo bioavailability and to support biowaivers also allows setting the dissolution specification and methods. The substitute of expensive clinical trials with the use of IVIVC is perhaps the most important feature of IVIVC. From the regulatory point of view IVIVC can assist certain scale-up and post-approval changes. IVIVC principles have been mostly applied to oral products, there exists a need to develop methodologies and standards for non-oral delivery systems, to develope more meaningful dissolution and permeation methods.

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