

Establishment of *in vivo* - *in vitro* Correlation: a Cogent Strategy in Product Development Process

Sharwaree Hardikar*, A V Bhosale and R N Budhawant

Department of Pharmaceutics, Pune District Education Association's Seth Govind Raghunath Sable College of Pharmacy, Saswad. Tal- Purandhar, Dist.- Pune 412301. India

ABSTRACT

Background: In vivo In vitro correlation (IVIVC) development and its validation is an extremely important phase of dosage form (new as well as generic) optimization. It is a liaison (preferentially linear) between a suitable pharmacokinetic parameter (peak plasma drug conc. i.e. C_{max}, time for peak plasma drug conc. i.e. t_{max} and Area under the Curve i.e. AUC) and in vitro characteristics. **Need:** During optimization modifications in formulation composition, manufacturing method, required equipments or even in batch size. If these changes are made, in vivo bioavailability study in healthy human volunteers may be required to prove bioavailability of newly developed dosage form with the earlier one. These facts halt the marketing of new formulation with added cost. Thus, it would be enviable to develop in vitro test that corresponds to bioavailability data. **Applications:** Thus the main purpose of development of IVIVC is to replace in vivo bioavailability and to support bio waivers. As IVIVC comprises linkage between suitable in vivo pharmacokinetic parameter to in vitro characteristic pertaining to dissolution; it can also be used to set up dissolution specifications and/ or validation of methods of dissolution studies. It also assists as means of quality control for certain SUPAC (Scale Up and Post Approval Changes). Moreover there must be some in vitro means to assure batch to batch consistency during large scale manufacturing. After successful establishment of IVIVC; dissolution method used in such establishment is used as routine quality control test as a measure of physiological performance of drug product.

Keywords: *In vivo In vitro correlation*, Biowaiver, Deconvolution, Convolution.

INTRODUCTION

Formulation development is critical stage of entire product development process. Establishment of *In vivo In vitro correlations* (IVIVC) is an integral part of formulation development and optimization. The overall tenure involved in momentous institution of IVIVC is depicted in (Figure 1).

IVIVC is a mathematical relationship between *in vitro* properties (dissolution) of the dosage form to its bioavailability. IVIVC reduces the number of clinical trials required in prop up of regulatory approval of a new product and during SUPAC (Scale-Up and Post Approval Changes). Regulatory guidance was developed by FDA and referred as IVIVC Guidance.^{1,2}

Thus, IVIVC plays a key role in product development.^{1,3} As it Serves as a replacement of *in vivo* and helps to support biowaivers (particularly in case of BCS class I drugs), Use full in selection of dissolution schemes and specifications and their eventual validation, Assists in assessment of quality of the product during manufacturing and Useful in optimizing formulations.

Establishing the Dissolution Specifications

From the preclinical development until proof of concept initiation for design of dosage form, pharmacokinetic testing is often carried out. Pharmacokinetics of different physical forms, salts, variable particle sizes and more than one formulations of

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Address for

correspondence:

Sharwaree Hardikar

Pune District Education

Association's Seth Govind

Raghunath Sable College

of Pharmacy, Saswad.

Tal- Purandhar, Dist. Pune

412302.

Phone/Fax: 02115-222212

E-mail: sharwareehardikar@

gmail.com



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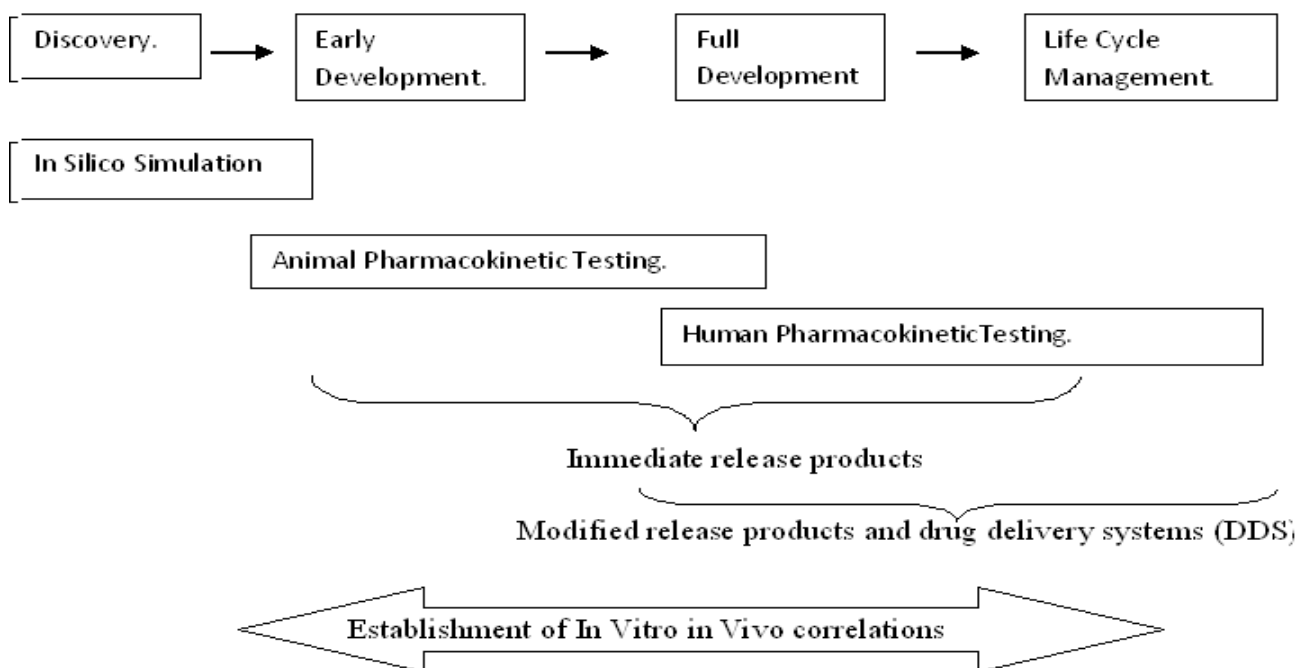


Figure 1: IVIVC in Drug Development Process.

drug molecule can be evaluated in a preclinical animal model. This is the first time when *in vitro* measurement (i.e. dissolution profile) is correlated to its *in vivo* performance such as C_{max} and Area under the Curve (AUC). At this stage, bio relevant dissolution method is developed. Bio relevant dissolution method involves bio relevant dissolution media that are virtually same as biological fluids. Use of such media assists successful establishment of IVIVC.^{4,5}

Generic products are often developed by using new excipient which is different than used in branded product. Not only excipient but manufacturing process may differ significantly. Hence dissolution methods and specifications for branded product may not be suitable for generic products; though they are bioequivalent. A preclinical pharmacokinetic study (bioavailability studies in animal) for such generic products (even for newly developed product) may be used to develop appropriate dissolution method.⁴ IVIVC establishment using human pharmacokinetic data has scientific and practical value. This further supports future formulation development during life cycle management.

Generally dissolution test is employed to assess the impact of formulation and processing variables on drug release characteristics. It is also the primary evaluation parameter that can be correlated with bioavailability of drug under consideration. Thus correlation in some form is desirable between suitable *in vitro* dissolution characteristics e.g. $t_{50\%}$, $t_{90\%}$ etc. and relevant *in vivo* pharmacokinetic parameter e.g. t_{max} ; which is

referred as IVIVC. Developing a dissolution test with an aim to establish such relationship is an ever challenging job of a formulation development pharmacist/researcher in the overall development process of a drug product. Biopharmaceutics Classification System (BCS) guides researcher in deciding the terms under which the IVIVCs are anticipated (table 1). A decision tree for dissolution testing method/design based on BCS and physicochemical properties of the molecule have been proposed (Figure 2, 3).⁶⁻⁸

Development of *in vitro in vivo* Correlations

Tactical development of such relation is two stage programme⁹

IVIVC establishment is a vivacious process and commences from the initial period of product development. Clinical phase trials and formulation development goes contemporarily to save the time involved in overall product development process.

An assumption of IVIVC i.e. appropriate *in vitro* dissolution targets to be predefined to meet the desired *in vivo* bioavailability specifications assuming level A. This correlation hypothetical pharmacokinetic/pharmacodynamic model is subject to revision as the prototype formulation is analyzed for *in vivo* behavior. Usually more than one prototype formulations within one technology are tested with the dissolution methodology and technology available in the laboratory. Thus this activity culminates in a pilot pharmacokinetic study. The results of this pilot pharmacokinetic study result in retrospec-

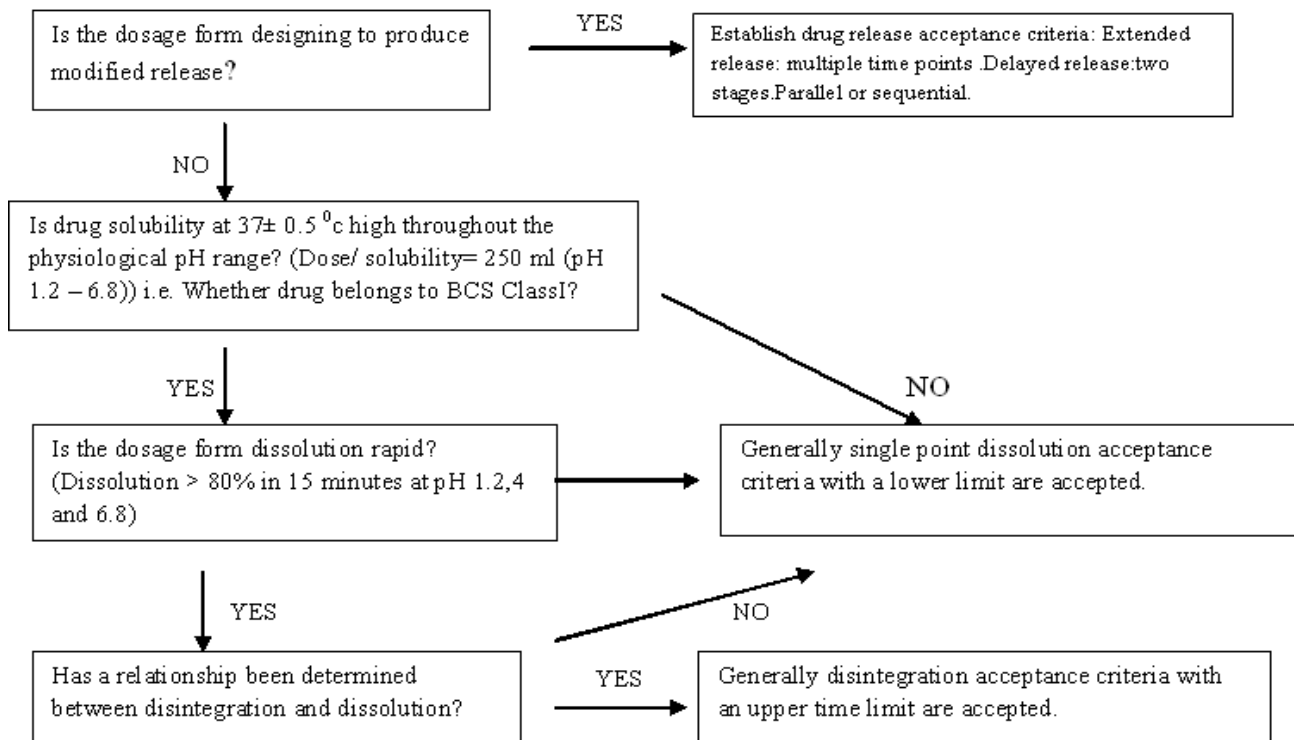


Figure 2. A Decision Tree for Dissolution Testing Based on BCS for Appropriate Type of Drug Release Acceptance Criteria for IVIVC.

Class	Solubility	Permeability	Dissolution Methodology
I	High	High	Single point if NLT 85 % Q in 15 min Multiple point if Q < 85 % in 15 min
II	Low	High	Multiple point
III	High	Low	Same as Class I
IV	Low	Low	Same as Class II

Multiple Point Test: 4 - 6 points each test

Test 1: pH=1, 2 hr., Volume=250 ml

Test 2: Media Change at 0.5, 1, 2 hr. to pH 4.5, 6.5, 8.0

Surfactant media when required to achieve Q=85 %, Volume=900 ml

Figure 3. Dissolution Methodology for Immediate Release Products.

tive IVIVR. This work many times steers the process of resetting of *in vitro* target revision of formulation strategy.

Once defined formulation is developed that meets the *in vivo* target; the next aim is to progress for its approval through optimization. Optimized formulation is one which exhibits drug release profile that reveals mecha-

nism of release. A release rate controlling parameter is identified from the retrospective data generated in first stage. In second stage extensive *in vitro* dissolution study is performed across dissolution media of using variable pH and various apparatus for optimized formulation. This leads to execution of prospective IVIVR study which is useful further in quality control method devel-

opment. This is ideally level A IVIVC but multiple levels C IVIVC is acceptable.

Thus a typical IVIVC development involves three essential elements, (i) model development, (ii) model validation and (iii) model application. To develop a meaningful IVIVC depends upon the design of the delivery system, its composition, manufacturing process, properties of the drug and the method of dissolution.⁵

IVIVC Model Development

Five correlation levels are defined in the FDA guidance viz. IVIVC level A, IVIVC level B, IVIVC level C, IVIVC level D, and IVIVC multiple level C.¹⁰ The level of correlation is decided from the propensity of IVIVC to reflect the complete plasma drug level–time profile. In the process of IVIVC development, the *in vitro* drug release parameters which resemble *in vivo* drug performance are identified. The suitable design of *in vitro* dissolution tests, capable of differentiating between the formulations with dissimilar bioavailability, plays a key role in the certainty of the IVIVC. Therefore, it is necessary that *in vitro* dissolution tests precisely reflect *in vivo* conditions, when they are used to set up an IVIVC.

IVIVC at “Level A” This is the most common type of correlation observed in new drug applications (NDAs) submitted to the FDA. From regulatory perspectives, it is considered to be the most useful. It is the most acceptable level of correlation. This level is accepted by regulatory authorities because of existence of point to point correlation during the time course between *in vivo* and *in vitro* profiles. The establishment of a robust Level A correlation involves adaption of either deconvolution or convolution method.

Deconvolution Methods

For developing IVIVC Level A, that can be accepted by regulatory agencies, an IVIVC model needs to be developed for at least two formulations, with three or more being preferred. The assessment of *in vivo* absorption contour from the concentration–time data can be achieved through the three stage deconvolution methods involving Wagner–Nelson (WN), Loo–Riegelman (LR) methods or model independent methods. The model-dependent Wagner–Nelson method is derived by adopting one-compartment model; it has an advantage that it demands plasma profile of the drug after oral administration only. The Loo–Riegelman method needs intravenous data and is derived by adopting two-compartment model. *In vivo* plasma data from an oral solution/intravenous dosing is essential for the application of model-independent numerical deconvolution.

The *in vitro* dissolution profiles are scaled by mathematical means, as there are intrinsic differences *in vitro* disso-

lution specifications and *in vivo* biological conditions and represented by equation 1 and is referred to as ‘deconvolution method’⁴.

$$X_{\text{vivo}}(t) = A_1 + A_2 \times X_{\text{vitro}}(B_1 \times B_2 \times t) \text{ if } t > T \text{ then } t = T \dots\dots\dots \text{Eq.}^n \text{ 1.}$$

$$\text{if } B_1 < B_2 \times t \text{ then } B_1 + B_2 \times t = 0$$

Whereby, $X_{\text{vivo}}(t)$ represents the absorption profile as a function of time and $X_{\text{vitro}}(t)$ represents the dissolution profile. The modifications to the *in vitro* profile as a function of time t is achieved by introduction of a time scale factor B_2 . If the *in vitro* dissolution process occurs faster or slower than the corresponding *in vivo* dissolution, the correction factors are added. To allow an initial lag time in the *in vivo* absorption (because of necessary pre absorption transit through the stomach) through a lag time B_1 , and through a cut-off factor T to accommodate dissolution slower than the GI transit times. The actual correlation is obtained via comparison of the *in vivo* profile with the slope A_2 and y-intercept A_1 as a link function between both profiles.

Determining the Fraction of Dose Absorbed (For determining $X_{\text{vivo}}(t)$):¹¹

1. Wagner Nelson method - This is predictive mathematical equation for establishment of IVIVC of drug following first order kinetics in one compartment model.

$$F_{(t)} = C_t + \frac{K_{el} AUC_0^t}{K_{el} AUC_0^\infty} \dots\dots\dots \text{Eq.}^n \text{ 2.}$$

Where $F_{(t)}$ is fraction bioavailable (absorbed), C_t is concentration of drug at time t in blood / plasma, drug’s elimination rate constant at time t is K_{el} and AUC is Area under the Curve. This equation is useful to transform C_t values to values as first step of deconvolution method.

2. Loo–Riegelman method: It is useful in a drugs following two compartment models and obeying first order kinetics.

$$F_{(t)} = \frac{C_t + K_{el} AUC_{(0-t)} + (X_p)_t / V_c}{K_{el} AUC_{(0-\infty)}} \dots\dots\dots \text{Eq.}^n \text{ 3.}$$

C_t and $AUC_{(0-t)}$ are calculated from the plot of C_t vs. t and the values of $(X_p)_t / V_c$ can be approximated by Loo–Riegelman method. K_{el} is elimination rate constant of a drug and AUC is Area under the Curve.

3. Model independent methods or numerical deconvolution method: It is particularly useful when the drug follows nonlinear kinetics.

Convolution Methods:⁴

Conversion of the dissolution contour to a plasma concentration contour can take place via convolution (input to output). Recently, convolution methods have been

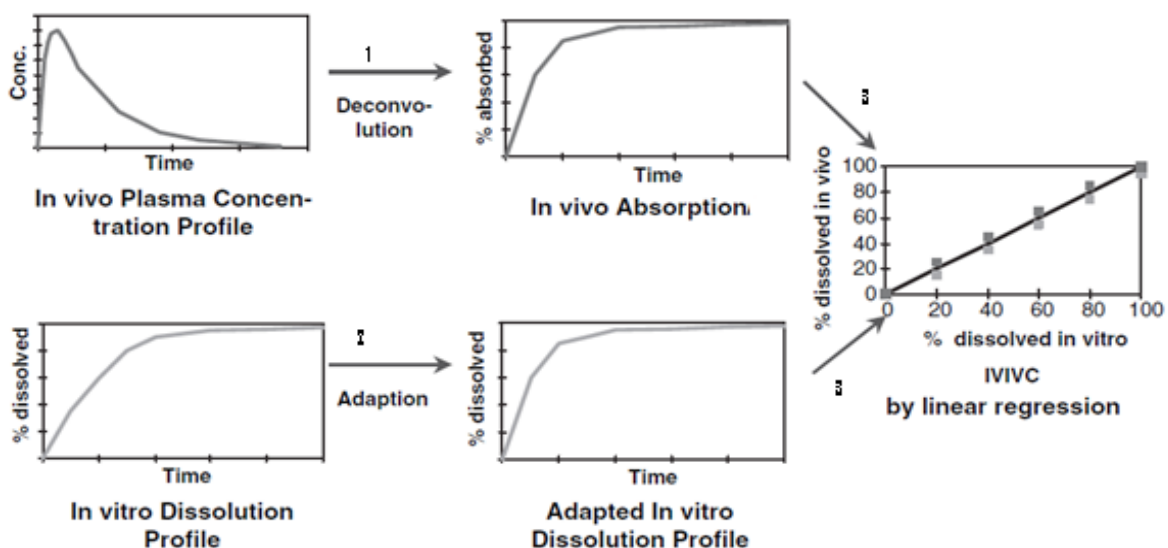


Figure 4. Classical Three Steps of Deconvolution.

established, which convolutes the dissolution contour without applying the correlation of the absorption/*in vivo* dissolution contour with the dissolution profile *in vitro* (i.e., physiology based model and simulation software). The model uses multiple differential equations representing various physiological events and convolution-based methods. Such a convolution does not take place in several partial stages, rather in one single stage that is schematically illustrated in (Figure 4 and 5). A major advantage of convolution-based methods for IVIVC is that no additional *in vivo* data such as intravenous injections or oral solutions are required. However, these methods can only mathematically fit the data by minimizing the squared error; even though the results

obtained are mathematically correct it may not be meaningful PK or physiological models. A critical assessment of the calculated parameters is absolutely necessary. Further, the fitting procedure should be performed several times with different starting values, in order to avoid reaching a local minimum. Last but not the least, these methods should be optimized to as few variables as possible, as the fitting procedure becomes more complex and error-prone with more variables.

IVIVC at “Level B” If the entire profile cannot be used for correlation, reduction in *in vivo* and the *in vitro* data that is comparable is required. The model-independent statistical moment theory uses empirical distribution functions for the purpose. Level B correlation is

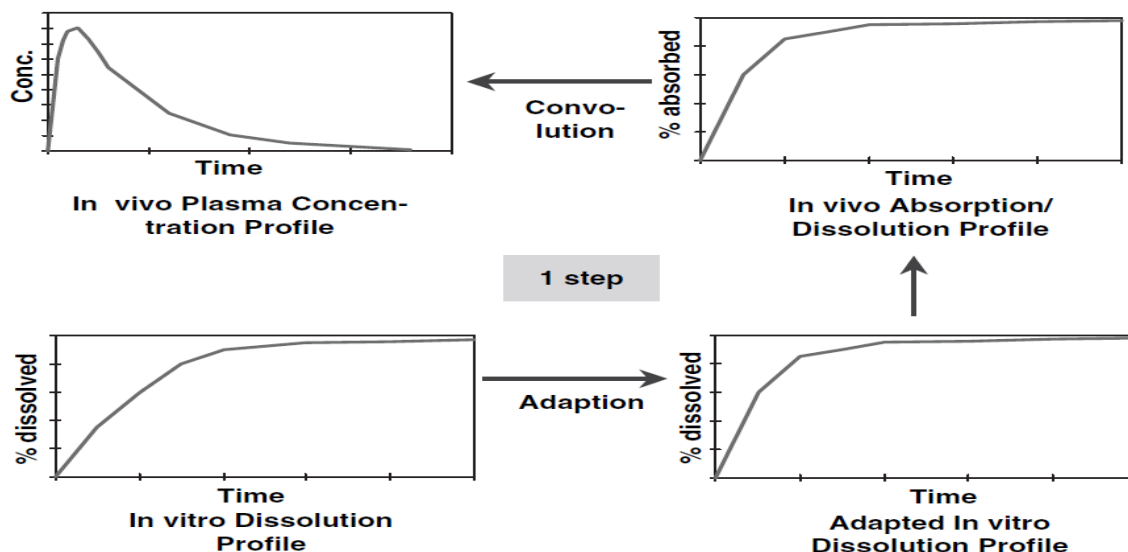


Figure 5. The One-Step Procedure of a Convolution.

established by comparing the mean *in vitro* dissolution time to either mean residence time (MRT-*in vivo*) or the mean dissolution time *in vivo* of the product. Level B correlation is not point to point correlation. That is why it never replicates the *in vivo* plasma level actually.

IVIVC at “Level C” It ascertains a single point relationship between *in vitro* dissolution parameter and a pharmacokinetic parameter. Any dissolution time point is generally compared (e.g, $t_{90\%}$) to one pharmacokinetic parameter (like AUC, t_{max} , and C_{max}). This is a very weak level of correlation, and has limited practicability in forecasting *in vivo* drug performance. Correlations at Level C means a lot during early phases of development of formulation.

IVIVC at “Multiple Level C” This relationship is almost equivalent to level A, and can be employed as a substitute to the Level A correlation. It relates relevant pharmacokinetic parameter to the drug’s quantity dissolved *in vitro* at respective time points. It can be employed to defend biowaiver(s) if the correlation is established over the entire dissolution contour with one or more pharmacokinetic parameters of significance. If such correlation is possible, then “Level A” correlation is also possible.

IVIVC at Level D: It is a nonparametric rank order correlation between the *in vitro* dissolution parameter and an *in vivo* pharmacokinetic parameter. It is usually based on ordinal (but not quantitative) data, thus considered to be the weakest correlation.

Thus IVIVC is defined as^{12,13}

United State Pharmacopoeia (USP) definition: The establishment of a sensible relationship between a physiological performance, or a parameter derived from a physiological performance produced by a dosage form, and a distinctive physicochemical performance or property of the same dosage form (US Department 1995).

Food and Drug Administration (FDA) definition: It is a predictive mathematical model relating the relationship between an *in vitro* performance parameter of a dosage form and it’s *in vivo* response parameter. Generally, the *in vitro* performance parameter is the rate and extent of drug dissolution or release and the *in vivo* response is the drug concentration in plasma or quantity of drug absorbed (Center for Drug Evaluation, USFDA 1997).

IVIVC MODEL VALIDATION^{14,15}

For IVIVC, minimum 12 individual dosage units from every formulation batch have to be tested for their dissolution profiles. Bioavailability studies should be completed with that number of subjects which is sufficient to demonstrate the performance of the test formulation adequately. BCS i.e. Biopharmaceutical Classification System is useful to decide the condition under which the predictable IVIVC is expected.

The IVIVC is developed with an aim to predict the plasma conc. time profile. It is, thus very important to judge the predictive concert by calculating its prediction error. Based on the proposed application of the IVIVC and the drug’s therapeutic index justification of internal /external predictability may be is done.

It is an estimation of predictive performance of the model proposed during strategic development of the correlation. Prediction error is evaluated considering the proposed application of IVIVC and drug’s therapeutic index. Since, IVIVC could be used to anticipate *in vivo* performance in case of any future change in the formulation, external predictability is essential. External predictability depends on the supplementary data sets (strategic development of IVIVC step 2) and internal predictability depends on initial data of step 1 of strategic development of IVIVC.

Table 1. IVIVC Probabilities for Immediate Release Products.

CLASS	Solubility	Permeability	Absorption rate controlling parameter	For immediate release product
I	High	High	Gastric emptying	IVIVC may be expected; if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlations.
II	Low	High	Dissolution	IVIVC may be expected; if <i>in vitro</i> rate of dissolution is equivalent to <i>in vivo</i> dissolution rate (except when the dose is very high).
III	High	Low	Permeability	Absorption (Permeability) is slow and difficult to establish IVIVC.
IV	Low	Low	Case to Case	Limited / no IVIVC is expected.

Evaluation of Predictability of IVIVC

Prediction error (% P.E) C_{max} and AUC can be calculated as-

$$\% \text{ P.E} = \frac{(\text{observed-predicted}) \times 100}{\text{Observed}} \dots\dots\dots \text{Eq.}^n 4.$$

Internal Predictability

PDE i.e. Percent Prediction Error (average) of $\leq 10\%$ establishes predictability. Percent Prediction Error (PDE) for any formulation should not be $> 15\%$. Non compliance with these criteria depicts inconclusive internal predictability and external predictability should be performed.

External Predictability

Percent Predictability Error $\leq 10\%$ for C_{max} and AUC sets up the external predictability. Percent predictability error between 10–20% shows inconclusive predictability. In such cases further study is demanded using additional data sets. Percent predictability error $> 20\%$ generally indicates poor predictability, and has to be justified.

IVIVC Model Application^{16,17}

The development and validation of *In vivo In vitro correlation* (IVIVC) is even more imperative stage of product (new / generic) optimization. It is a linear correlation between *in vitro* characteristics and a biological parameter (C_{max} , t_{max} , AUC).

Optimization may require modification formulation composition, manufacturing method, required equipments or even in batch size. If these changes are made, *in vivo* bioavailability study in healthy human volunteers may be required to prove bioavailability of newly developed dosage form with the earlier one. These facts halt the marketing of new formulation with added cost. Hence, it would be inevitable to build up *in vitro* test that represents bioavailability. Thus this is main point of developing and establishing IVIVC. Once established it serves as proxy to *in vivo* bioavailability and props up bio waivers. Since IVIVC includes institution of relationship between *in vivo* parameter (bioavailability) to *in vitro* parameter (dissolution); it can also be utilized to ascertain dissolution specifications and/or authenticate/validate the dissolution testing methods. It also assists as quality control parameter for certain SUPAC i.e. scale up and post approval changes. Moreover there must be some *in vitro* means to assure batch to batch consistency during large scale manufacturing. After successful establishing IVIVC; dissolution method used in such establishment used as routine quality control test as a measure of physiological performance of drug product.

CONCLUSION

While FDA guidelines for establishment of IVIVC are applicable to oral dosage forms; these principles can be used in design and development of non oral dosage forms also. IVIVC's are applicable for granting the bio-waivers, deciding dissolution specifications, setting release rate specifications. A prophetic correlation of level A facilitates the process of proposal as biowaiver formulation. This is so, as the *in vitro* dissolution substitutes the *in vivo* bioavailability of the formulation. Therefore, a meaningful and predictive correlation can be a useful tool both in product development and regulatory decision making.

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CONFLICT OF INTEREST

Nil

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