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## **AN OVERVIEW ON IVIVC AND DISSOLUTION METHODOLOGY**

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### **ABSTRACT**

The concept and application of the in vitro-in vivo correlation (IVIVC) for pharmaceutical dosage forms have been a main focus of attention of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. An IVIVC should be evaluated to demonstrate that predictability of in vivo performance of a drug product from its in vitro dissolution characteristics is maintained over a range of in vitro dissolution release rates and manufacturing changes. Certainly, implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop in vitro tests that reflect bioavailability data. The FDA Guidance on IVIVC provides general methods is considered as an important complimentary tool for use in in vitro dissolution techniques, allowing more information to be collected about drug behaviour in various GI conditions & in which all drug of high dosage can be easily administered. The ADS method is sensitive, effective & reliable in predicting in vivo performance of dosage forms. The number of studies reported in the area of establishing IVIVCs for non-oral dosage forms are very scarce and further research is necessary in the development of more meaningful dissolution and permeation methods.

## INTRODUCTION

Pharmaceutical industries are hungry for rapid drug development & approval while regulatory agencies need assurance of product quality & performance. This necessitates the research to find out link between dissolution testing to prediction of bioavailability<sup>(1-2)</sup>.

In 1980s attention was focused towards in –vitro in-vivo correlation (IVIVC). A workshop sponsored jointly by the USFDA industry concluded that the state of science & technology at that time did not permit meaningful IVIVC for Extended Release (ER) product on consistent basis .but encouraged further research in the area .Thereafter USP published a stimuli article indicating different levels of correlation. These levels are as Level A, Level B, Level C, Multiple Level C, and Level D Correlation.

The concept and application of the in vitro-in vivo correlation (IVIVC) for pharmaceutical dosage forms have been a main focus of attention of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in human healthy volunteers may be required to prove that the new formulation is bioequivalent with the old one. Certainly, implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop in vitro tests that reflect bioavailability data. A regulatory guidance for both immediate- and modified-release dosage forms has been, therefore, developed by the FDA to minimize the need for bioavailability studies as part of the formulation design and optimization. IVIVC procedures are specific to certain countries but could be adopted or used as the background for regulatory recommendations by other countries. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development. The main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers. IVIVCs could also be employed to establish dissolution specifications and to support and/or validate the use of dissolution methods. This is because the IVIVC includes in vivo relevance to in vitro dissolution specifications. It can also assist in quality control for certain scale-up and post-approval

changes, for instance, to improve formulations or to change production processes. There must be some *in vitro* means of assuring that each batch of the same product will perform identically *in vivo*. With the proliferation of modified-release products, it is essential to examine the concept of IVIVC in greater depth. Therefore, a more detailed article covering various aspects of an IVIVC study including complete process of developing the correlation with high quality, accurate and precise predictability, and identifying specific applications for such correlations might be of importance<sup>(3-5)</sup>.

Dissolution tests are one of the most important quality control tests in pharmaceutical analysis. A direct relationship has been demonstrated between *in vitro* dissolution rate of many drugs and their bioavailability and is generally known as *in vitro* - *in vivo* correlation, IVIVC. Dissolution is the process by which a solid solute enters a solution. In the pharmaceutical industry, it may be defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. Dissolution is considered one of the most important quality control tests performed on pharmaceutical dosage forms and is now developing into a tool for predicting bioavailability, and in some cases, replacing clinical studies to determine bioequivalence. Four basic types of dissolution apparatus including rotating basket (Apparatus 1), paddle method (Apparatus 2), reciprocating cylinder (Apparatus 3) and flow through cell (Apparatus 4) are specified by the USP and recommended in the FDA guidance. Dissolution behavior of drugs has a significant effect on their pharmacological activity. In fact, a direct relationship between *in vitro* dissolution rate of many drugs and their bioavailability has been demonstrated and is generally referred to as *in vitro-in vivo* correlation, IVIVC.

## **DEFINITIONS**<sup>(1-6)</sup>

The term correlation is frequently employed within the pharmaceutical and related sciences to describe the relationship that exists between variables. Mathematically, the term correlation means interdependence between quantitative or qualitative data or relationship between measurable variables and ranks. From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate *in vitro* release characteristics and *in vivo* bioavailability parameters. Two definitions of IVIVC have been proposed by the USP and by the FDA.

***United State Pharmacopoeia (USP) definition:***

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 <sup>1</sup>.

***Food and Drug Administration (FDA) definition:***

IVIVC is a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant 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.<sup>1</sup>

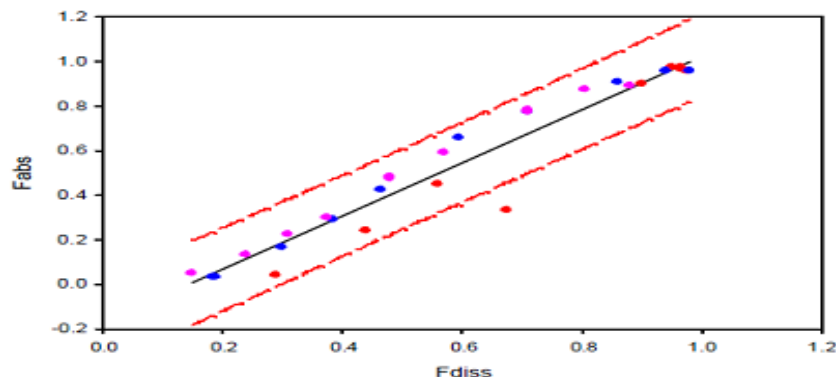
**CORRELATION LEVELS <sup>(7-8)</sup>**

Five correlation levels have been defined in the IVIVC FDA guidance. 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.

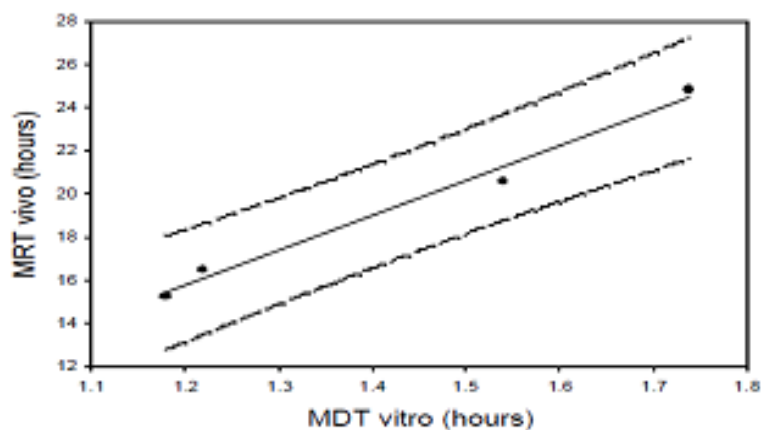
***Level A Correlation:***

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 (fig 1). Generally, percent of drug absorbed may be calculated by means of model dependent techniques such as Wagner-Nelson procedure or Loo-Riegelman method or by model-independent numerical deconvolution. These techniques represent a major advance over the single-point approach in that these methodologies utilize all of the dissolution and plasma level data available to develop the correlations and will be discussed more in detail later in this article.

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. In the case of a level A correlation, an in vitro dissolution curve can serve as a surrogate for in vivo performance. Therefore, a change in manufacturing site, method of manufacture, raw material supplies, minor formulation modification, and even product strength using the same formulation can be justified without the need for additional human studies. It is an excellent quality control procedure since it is predictive of the dosage form's in vivo performance.

**Fig 1 Level A Correlation****Level B Correlation:**

A level B IVIVC utilizes 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 (MDT vivo)(fig 2). Although a level B correlation uses all of the in vitro and in vivo data, it is not considered to be a point-to-point correlation, since there are a number of different in vivo curves that will produce similar mean residence time values. A level B correlation does not uniquely reflect the actual in vivo plasma level curves. Therefore, one can not rely upon a level B correlation alone to justify formulation modification, manufacturing site change, excipient source change, etc. In addition in vitro data from such a correlation could not be used to justify the extremes of quality control standards.

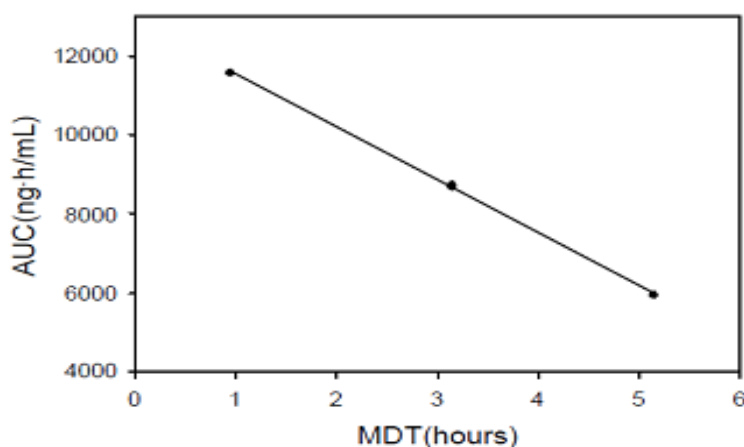
**Fig 2 Level B Correlation**

**Level C Correlation:**

In this level of correlation, one dissolution time point ( $t_{50\%}$ ,  $t_{90\%}$ , etc.) is compared to one mean pharmacokinetic parameter such as AUC,  $t_{\max}$  or  $C_{\max}$  (fig 3). Therefore, it represents a single point correlation and does not reflect the entire shape of the plasma drug concentration curve, which is indeed a crucial factor that is a good indicative of the performance of modified-release products. This is the weakest level of correlation as partial relationship between absorption and dissolution is established.

Due to its obvious limitations, the usefulness of a Level C correlation is limited in predicting in vivo drug performance. The usefulness of this correlation level is subject to the same caveats as a Level B correlation in its ability to support product and site changes as well as justification of quality control standard extremes. Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected. While the information may be useful in formulation development, waiver of an in vivo bioequivalence study (biowaiver) is generally not possible.

**Fig 3 Level C Correlation**

**Multiple-level C correlation:**

A multiple level C correlation relates one or several pharmacokinetic parameters of interest ( $C_{\max}$ , AUC, or any other suitable parameters) to the amount of drug dissolved at several time points of the dissolution profile. A multiple point level C correlation may be used to justify a biowaiver, provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A relationship should be demonstrated

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. If such a multiple level C correlation is achievable, then the development of a level A correlation is also 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.

***Level D correlation:***

Level D correlation is a rank order and qualitative analysis and is not considered useful for regulatory purposes. It is not a formal correlation but serves as an aid in the development of a formulation or processing procedure.

**DEVELOPMENT OF A CORRELATION** <sup>(9-12)</sup>

The most commonly seen process for developing a Level A IVIVC is to,

- (1) Develop formulations with different release rates, such as slow, medium, fast, or a single release rate if dissolution is condition independent (Fig.4)
- (2) Obtain in vitro dissolution profiles and in vivo plasma concentration profiles for these formulations (Fig.4)
- 3) Estimate the in vivo absorption or dissolution time course using an appropriate deconvolution technique for each formulation and subject (Fig. 4&5)

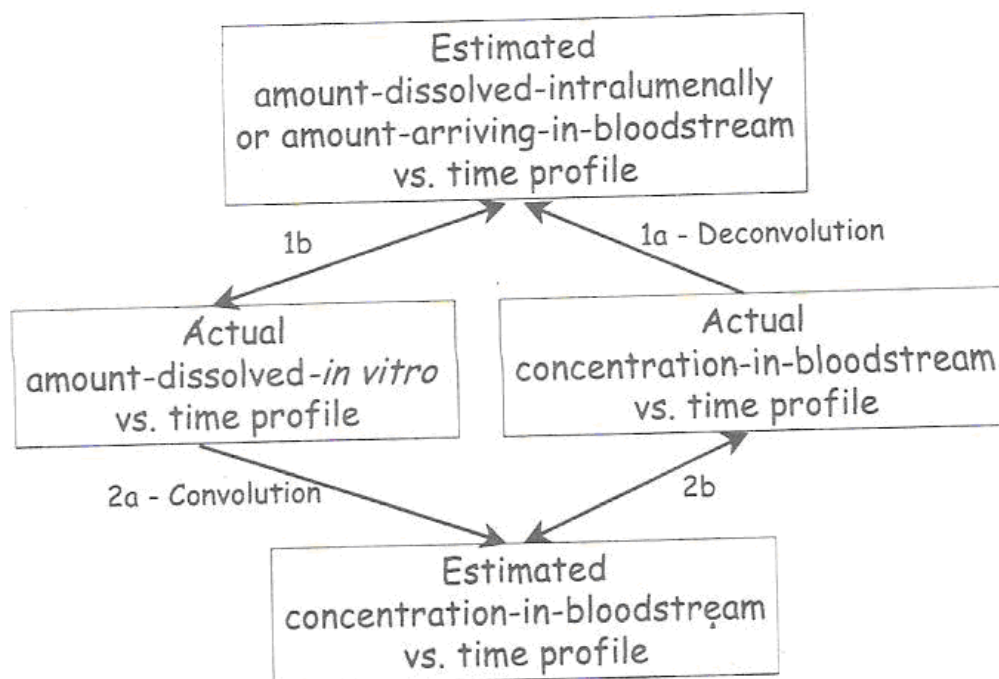
Point To Point IVIVCs can be established by using two approaches .The first approach is to establish a relationship between the actual time course of the in-vitro dissolution time course of the luminal dissolution or arrival in to the general circulation (fig.5), as mentioned by deconvolution of the observed concentration in the blood stream v/s time profile. The second approach is to establish relationship between the observed time course of plasma drug concentration and time course of plasma level (fig.5) estimated by convolution of the in-vitro dissolution data.

### Development of IVIVC: level A

*Two steps approach*
*One step approach*

<p>➤ develop formulations with different release rates, such as slow, medium, fast;</p> <p>➤ obtain in vitro dissolution profiles and in vivo plasma concentration profiles for these formulations</p>	
Step 1	<p>▪ estimate the <i>in vivo absorption or dissolution time course</i> using an appropriate technique for each formulation and subject.</p>
Step 2	<p>▪ Establish the Link model between in vivo and in vitro variable</p> <p>▪ Predict plasma concentration from in vitro data using the Link model</p>
<p>▪ Predict plasma concentration from in vitro profile using a LINK model whose parameters are fitted in one step</p> <p>• Do not involve deconvolution</p> <p>• Link model is not determined separately</p> <p>• Can be done without a reference (IV bolus, oral solution or IR form)</p>	

**Fig 4 Development of level A Correlation**



**Fig 5 Approaches For Developing Point To Point IVIVC.**



One method to develop level A correlation is to estimate the *in vivo* absorption or dissolution time course using an appropriate deconvolution techniques such as Wagner-Nelson procedure or Loo-Riegelman method or numerical deconvolution for each formulation and subject. Wagner-Nelson and Loo-Riegelman methods are both model dependent in which the former is used for a one-compartment model and the latter is for multi-compartment system. The Wagner-Nelson is less complicated than the Loo-Riegelman as there is no requirement for intravenous data.

Deconvolution principle was used to evaluate the extent of absorption and first pass elimination of selected drugs. Use of deconvolution theory to delineate the absorption and gut wall metabolism of drugs. This theory provides an advantage for those drugs for which multiple sites of measurement are restricted and for experimental protocols that do not allow for extended blood sampling. Also, no compartmental model specification is required. The theory was used to generate an estimate of orally absorbed drug.

The convolution principle may be expressed as follows:

$$C_{(t)} = C_{(\delta)} * f_{(t)} \quad \dots(1)$$

The response function,  $C_{(t)}$ , is obtained by convolution of the unit impulse function,  $C_{(\delta)}$ , with the input function,  $f_{(t)}$

Mathematical convolution has been functionally expressed by asterisk.

Deconvolution is the mathematical inverse of convolution.

The literature reported values of *in vitro* study, obtained using a discriminative and biorelevant test methodology, were used to generate the calculated plasma concentration of a drug. The volume of distribution, elimination rate constant and the dose of the drug are needed to predict the calculated plasma concentration. For validating the proposed equation, Wagner-Nelson method was adopted to generate the values of elimination rate constant and volume of distribution in a literature reported *in vivo* data set. The applications of the rearranged form of the Wagner-Nelson equation are shown in judging *in vitro in vivo* correlation and also in dosage form design. The steps for obtaining the rearranged form of the Wagner-Nelson equation are given in an appendix. The proposed method can be used if the custom made computer programs are not available.

An *in vitro in vivo* correlation (IVIVC) deals with a relationship (preferably linear) between an *in vitro* characteristic (e.g. *in vitro* drug dissolution) and a biological parameter (maximum

plasma drug concentration ( $C_{\max}$ ), time at which  $C_{\max}$  reach ( $t_{\max}$ ) or area under the curve). The FDA guidance document states that the main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for in vivo bioavailability study<sup>1</sup>. The IVIVC is used in the formulation development work and also in scale up and post approval changes (SUPAC). The four categories of *in vitro in vivo* correlation described in the FDA guidance document are Level A, Level B, Level C and multiple Level C. Out of these four categories, level A correlation is the most common type of correlation observed in new drug application (NDA), since it represents a point-to-point relationship between *in vitro* drug dissolution and *in vivo* bioavailability of the drug from a dosage form.

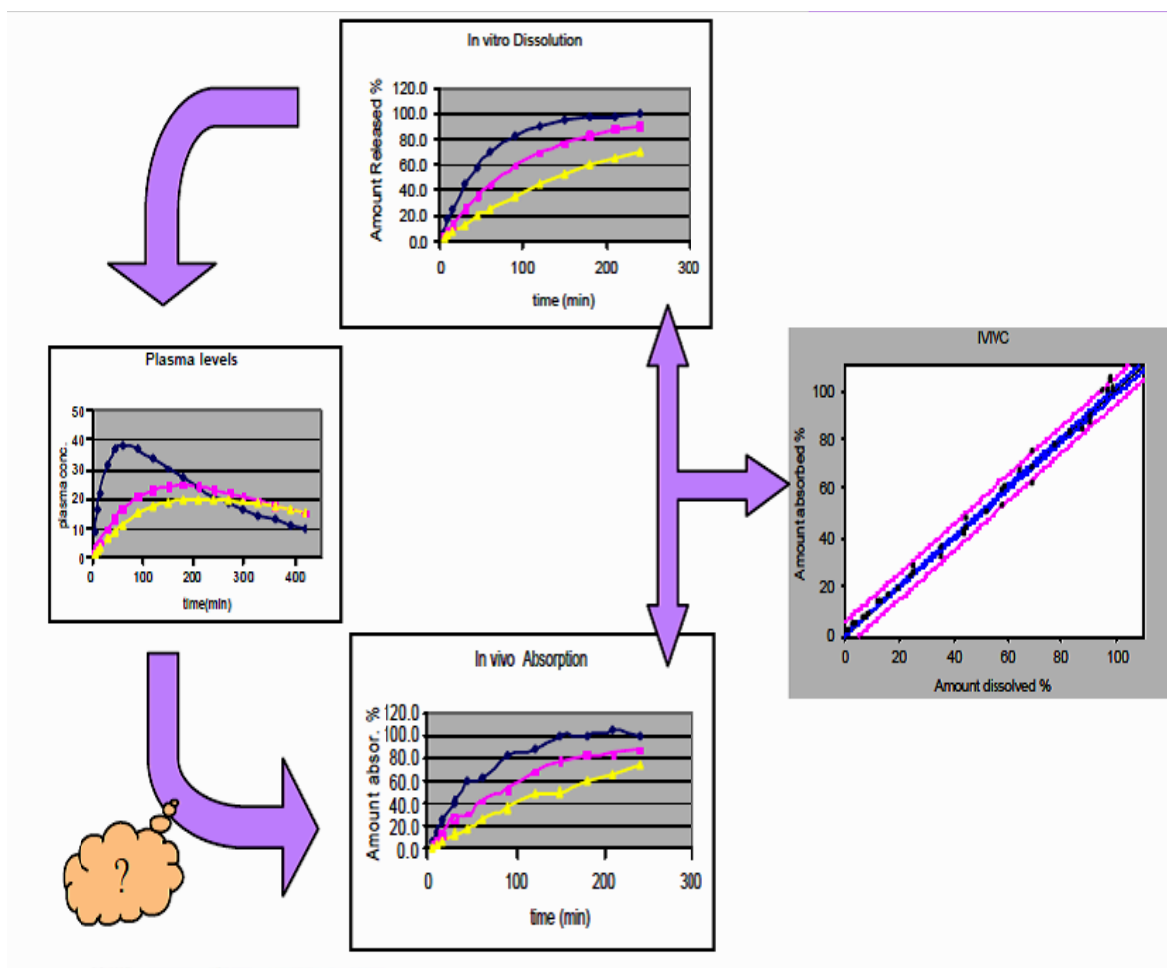
Mojaverian et al. stated that it is possible to obtain IVIVC by deconvoluting the plasma concentration-time curve using a model independent method such as Wagner-Nelson method or direct mathematical deconvolution and time correction factor<sup>2</sup>. Even though there are numerous examples of IVIVCs in the literature, many of the correlation have not been rigorously tested through a systematic evaluation of their predictability and majority of them are written by keeping well experienced pharmacokineticians in mind, who are having an access to sophisticated custom made computer programs. Balan et al., used "Kinetica" Software (version 2.0.2, Innaphase, France) for the establishment of Level A correlation.<sup>3</sup> Rossi et al., carried out simulations using a computer program to generate plasma concentration-time profile for diltiazem HCl, whose disposition obey to an open two-compartment model.

Wagner and Nelson developed an equation (see Appendix - I) for calculating absorption rate constant ( $K_a$ ) and fraction of dose absorbed from plasma drug concentration time profile (in vivo data) for open-compartment model<sup>4</sup>. The Wagner-Nelson method does not require a model assumption concerning the absorption process. Mathematical calculation of fraction of dose absorbed at various time points, from given plasma concentration versus time profile, has been demonstrated very well in literature. The *in vitro in vivo* correlation is generated using pooled mean fraction of dose dissolved (FRD) and pooled mean fraction of dose absorbed (FRA) from two or more formulations. The objective of the present study is to present a rearranged form of the Wagner-Nelson equation for evaluating IVIVC. The Loo-Riegelman method requires drug concentration time data after both oral and intravenous administration of the drug to the same subject and the fraction absorbed at any time  $t$ .

### **Development of Level A correlation** <sup>(12-14)</sup>

Alternative approaches to developing Level A IVIVCs are possible.

1. The IVIVC relationship should be demonstrated consistently with two or more formulations with different release rates to result in corresponding differences in absorption profiles. Although an IVIVC can be defined with a minimum of two formulations with different release rates, three or more formulations with different release rates are recommended. Exceptions to this approach (i.e., use of only one formulation) may be considered for formulations for which in vitro dissolution is independent of the dissolution test conditions (e.g., medium, agitation, pH).
2. Ideally, formulations should be compared in a single study with a crossover design.
3. If one or more of the formulations (highest or lowest release rate formulations) does not show the same relationship between in vitro dissolution and in vivo performance compared with the other formulations, the correlation may still be used within the range of release rates encompassed by the remaining formulations.
4. The in vitro dissolution methodology should adequately discriminate among formulations. Dissolution testing can be carried out during the formulation screening stage using several methods. 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.
- 5 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.
6. Time scaling may be used as long as the time scaling factor is the same for all formulations. Different time scales for each formulation indicate absence of an IVIVC.



**Fig 6 Development of Level A Correlation**

### IMPORTANT CONSIDERATIONS IN DEVELOPING A CORRELATION <sup>(14-18)</sup>

When the dissolution is not influenced by factors such as pH, surfactants, osmotic pressure, mixing intensity, enzyme, ionic strength, a set of dissolution data obtained from one formulation is correlated with a deconvoluted plasma concentration-time data set. To demonstrate a correlation, fraction absorbed in vivo should be plotted against the fraction released in vitro. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation. Under these circumstances, the correlation is considered general and could be extrapolated within a reasonable range for that formulation of the active drug entity.

In a linear correlation, the in vitro dissolution and in vivo input curves may be directly

superimposable or may be made to be superimposable by the use of appropriate scaling factor (time corrections). Time scaling factor should be the same for all formulations and different time scales for each formulation indicate absence of an IVIVC. Non-linear correlation may also be appropriate. In cases where, the dissolution rate depends on the experimental factors mentioned above, the deconvoluted plasma concentration-time curves constructed following administration of batches of product with different dissolution rates (at least two formulations having significantly different behaviour) are correlated with dissolution data obtained under the same dissolution condition. If there is no one-to-one correlation other levels of correlation could be evaluated. If one or more of the formulations (highest or lowest release rate formulations) may not illustrate the same relationship between in vitro performance and in vivo profiles compared with the other formulations, the correlation is still valid within the range of release rates covered by the remaining formulations. The in vitro dissolution methodology should be able to adequately discriminate between the study formulations. Once a system with most suitable discrimination is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation.

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. An established correlation is valid only for a specific type of pharmaceutical dosage form (tablets, gelatine capsules, etc.) with a particular release mechanism (matrix, osmotic system, etc.) and particular main excipients and additives. The correlation is true and predictive only if modifications of this dosage form remain within certain limits, consistent with the release mechanism and excipients involved in it.

Extrapolation of IVIVC established in healthy subjects to patients has to be taken into account. Drugs are often taken just before, with or after meal. All these factors may increase variability. A posterior correlation might be established using the patients' data only to increase the knowledge of the drug.

The release rates, as measured by percent dissolved, for each formulation studied, should differ adequately (e.g., by 10%). This should result in vivo profiles that show a comparable difference, for example, a 10% difference in the pharmacokinetic parameters of interest ( $C_{\max}$  or AUC) between each formulation.

**EVALUATION OF PREDICTABILITY OF IVIVC** <sup>(18-22)</sup>

An IVIVC should be evaluated to demonstrate that predictability of in vivo performance of a drug product from its in vitro dissolution characteristics is maintained over a range of in vitro dissolution release rates and manufacturing changes. Since the objective of developing an IVIVC is to establish a predictive mathematical model describing the relationship between an in vitro property and a relevant in vivo response, the proposed evaluation approaches focus on the estimation of predictive performance or, conversely, prediction error. Methodology for the evaluation of IVIVC predictability is an active area of investigation and a variety of methods are possible and potentially acceptable. A correlation should predict in vivo performance accurately and consistently.

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. Evaluation of internal predictability is based on the initial data used to define the IVIVC model. Evaluation of external predictability is based on additional test data sets.

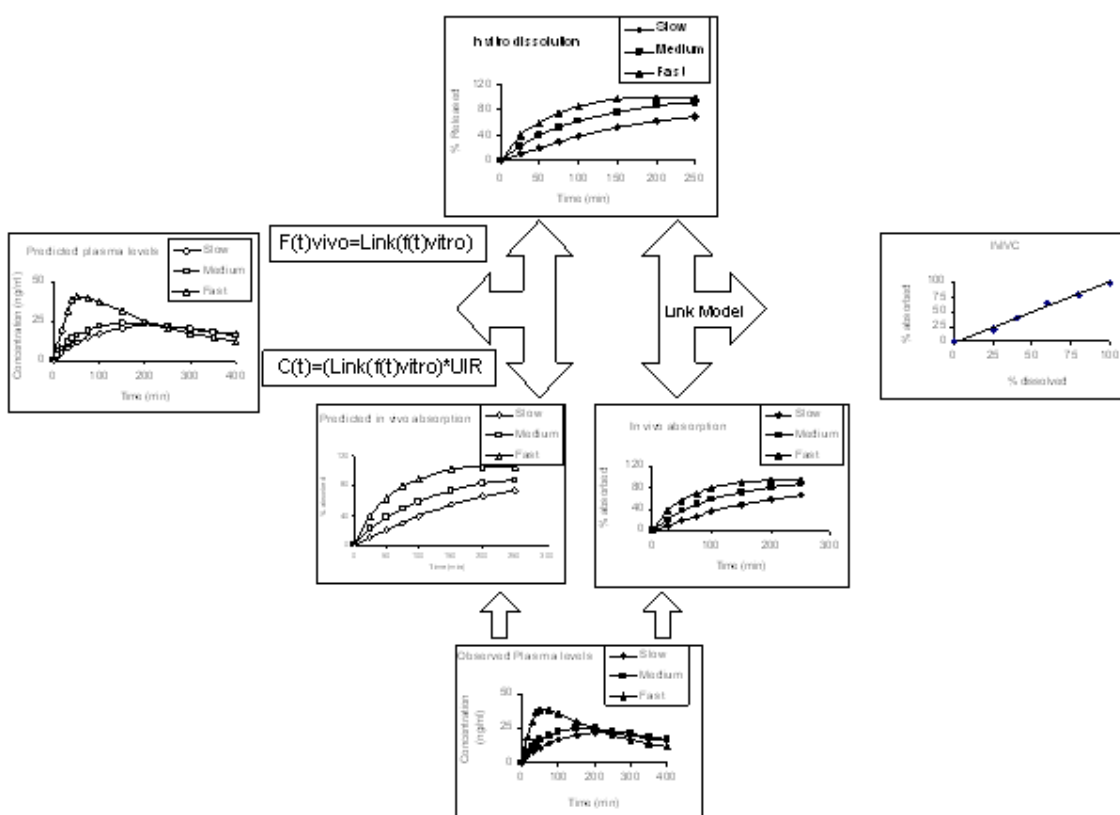
Internal predictability is applied to IVIVC established using formulations with three or more release rates for non-narrow therapeutic index drugs exhibiting conclusive prediction error. If two formulations with different release rates are used to develop IVIVC, then the application of IVIVC would be limited to specified categories for. Under these circumstances, for complete evaluation and subsequent full application of the IVIVC, prediction of error externally is recommended.

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. However, since the IVIVC will potentially be used to predict the in vivo performance for future changes, it is of value to evaluate external predictability when additional data are available.

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.

**Internal predictability:**

All IVIVCs should be studied regarding internal predictability. One recommended approach involves the use of the IVIVC model to predict each formulation's plasma concentration profile (or C<sub>max</sub> and/or AUC for a multiple Level C IVIVC) from each respective formulation's dissolution data. This is performed for each formulation used to develop the IVIVC model. Practically, in vitro dissolution rates is first estimated from dissolution data and is converted to in vivo dissolution rates by using the IVIVC model generated slope and intercept. If the cumulative drug release profile is sigmoid, then the Hill equation could be used to parameterize the in vitro drug release.



**Fig 7: Representative observed and predicted dissolution and plasma profiles which routinely used to develop and validate an in vitro in vivo correlation**

Where, %D = the percent drug dissolved at time t, D<sub>max</sub> = the maximum % drug dissolved, D50 = the time required for 50% of the drug to dissolve, t = time and γ = the sigmoidicity factor. In vitro release rates can be calculated by taking the first derivative of the Hill equation as listed below:

$$\%Dissolved = \frac{D_{max} \times t^r}{D_{50}^r + t^r} \dots (2)$$

The prediction of the plasma concentrations from the corresponding in vivo dissolution profiles is then accomplished by convolution of the in vivo dissolution rates and the pharmacokinetic model for the so called unit impulse response result from i.v. bolus data, oral solution or rapidly releasing (in vivo) immediate release dosage forms using equation In this equation symbols are as previously mentioned. The model predicted bioavailability is then compared to the observed bioavailability for each formulation. The percent prediction errors for Ct, Cmax or AUC could be determined as follows:

$$\% \text{ P.E.} = \frac{(\text{observed parameter} - \text{predicted parameter}) * 100}{\text{predicted parameter}} \dots (3)$$

Average absolute percent prediction error (% PE) of 10% or less for Cmax and AUC establishes the predictability of the IVIVC. In addition, the % PE for each formulation should not exceed 15%. If these criteria are not met, that is, if the internal predictability of the IVIVC is inconclusive, evaluation of external predictability of the IVIVC should be performed as a final determination of the ability of the IVIVC to be used as a surrogate for bioequivalence.

#### ***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. This involves using the IVIVC to predict the in vivo performance for a formulation with known bioavailability that was not used in developing the IVIVC model. % PE of 10% or less for C and AUC establishes the external predictability of an IVIVC. % PE between 10 - 20% indicates inconclusive predictability and the need for further study using additional data sets. Results of estimation of PE from all such data sets should be evaluated for consistency of predictability. % PE greater than 20% generally indicates inadequate predictability, unless otherwise justified.



**BIOAVAILABILITY STUDIES FOR DEVELOPMENT OF IVIVC<sup>(1-23)</sup>**

A bioavailability study should be performed to characterize the plasma concentration versus time profile for each of the formulation. Bioavailability studies for IVIVC development should be performed with sufficient number of subjects to characterize adequately the performance of the drug product under study. 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 reference product in developing an IVIVC may be an intravenous solution, an aqueous oral solution, or an immediate release product. 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. Drug absorption from GI tract following ingestion of an oral dosage form could be influenced by a number of in vivo variables.

For the determination of reproducible in vivo parameters and consequently useful in vitro in vivo relationship, it is imperative that such variables be identified. As a result, the study should be designed appropriately that as many variables as possible be eliminated or controlled to prevent or minimize their disturbance of IVIVC. Control or standardization of a number of variables including subject selection criteria such as age, gender, physical condition, etc., and the abstinence by the subject from coffee and other xanthenes containing beverages or food, alcohol, irregular diets and smoking before and during the study should be taken in to consideration. Food, posture and exercise may influence hepatic blood flow which in turn may substantially affect the absorption of drugs possessing high hepatic extraction ratio.

As pointed out earlier, one method to develop level A correlation is to estimate the in vivo absorption or dissolution time course using an appropriate deconvolution techniques such as Wagner-Nelson procedure or Loo-Riegelman method or numerical deconvolution for each formulation and subject. Wagner-Nelson and Loo-Riegelman methods are both model dependent in which the former is used for a one-compartment model and the latter is for multi-compartment system. The Wagner-Nelson is less complicated than the Loo-Riegelman as there is no requirement for intravenous data<sup>8</sup>. 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. According to Wagner-Nelson method, the

cumulative fraction of drug absorbed at time t is calculated from Equation 7 as follows:

$$F_T = \frac{C_T + K_E \int_0^T C dt}{K_E \int_0^T C dt} \quad \dots (4)$$

Where,  $C_T$  is plasma concentration at time T and  $K_E$  is elimination rate constant. The apparent absorption rate constant ( $K_a$ ) could be obtained from the least square fitted log-linear plot of the percent unabsorbed versus time. The absorption half-life ( $t_{1/2a}$ ) is calculated as  $0.693 / K_a$ .

The Loo-Riegelman method requires drug concentration time data after both oral and intravenous administration of the drug to the same subject and the fraction absorbed at any time t is given by,

$$F_T = \frac{C_T + K_{10} \int_0^T C dt + (X_p)_T / V_c}{K_{10} \int_0^T C dt} \quad \dots (5)$$

Where, in addition to symbols defined previously,  $(X_p)_T$  is the amount of drug in the peripheral compartment as a function of time after oral administration and  $V_c$  is the apparent volume of the central compartment.  $K_{10}$ , the apparent first order elimination rate constant of drug from the central compartment, is estimated from a previous or subsequent intravenous study of the same subject.  $(X_p)_T / V_c$  can be estimated by a rather complicated approximation procedure requiring both oral and intravenous data.

Deconvolution is a numerical method used to estimate the time course of drug input using a mathematical model based on the convolution integral. For example the absorption rate time course ( $r_{abs}$ ) that results in plasma concentration ( $c(t)$ ) may be estimated by solving the convolution integral equation for  $r_{abs}$ .

$$c(t) = \int_0^t c_{iv}(t-u) r_{abs}(u) du \quad \dots (6)$$

Where,  $c_{iv}$  represents the concentration time profile resulting from an instantaneous absorption of a unit amount of drug which is typically from bolus intravenous injection or reference oral solution data,  $c(t)$  is the plasma concentration versus time profiles of the tested formulations,  $r_{abs}$  is the input rate of the oral solid dosage form into the body and  $u$  is the variable of integration. Deconvolution method requires no assumptions regarding of the number of compartments in the model or the kinetics of absorption. Linear distribution and elimination are assumed. Like the Loo-Riegelman method, deconvolution requires data obtained after both oral and intravenous

administration in the same subject and assumes no differences in the pharmacokinetics of drug distribution and elimination from one study to the other. Drug concentrations must be measured at the same times following both oral and intravenous administration during the time that drug is absorbed after oral administration.

Mean residence time is the mean time that the drug resides in the body and is calculated by following equation:

$$MRT = AUMC / AUC \dots\dots(7)$$

Mean in vivo dissolution time reflects the mean time for drug to dissolve in vivo from a solid dosage form and is estimated as:

$$MDT_{solid} = MRT_{solid} - MRT_{solution} \dots\dots(8)$$

## APPLICATION OF AN IVIVC <sup>(23-25)</sup>

### 1. Biowaivers:

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.

### 2. Establishment of dissolution specifications:

It is relatively easy to establish a multipoint dissolution specification for modified-release dosage forms. The dissolution behaviour of the biobatch may be 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. The FDA guidance describes the procedures of setting dissolution specifications in cases of level A, multiple level 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<sub>max</sub> and AUC. Predicted plasma concentration and consequent AUC and C<sub>max</sub> could be calculated using convolution or any other appropriate modeling techniques. 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  $\pm 10\%$  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  $\pm 10\%$  (a total of 20%) or less of the labelled 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.

#### **DISSOLUTION MEDIA AND METHODOLOGY** <sup>(25-27)</sup>

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 solubilisation of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. The solubility of a drug is determined by dissolving the highest unit dose of the drug in 250 ml of buffer adjusted between pH 1 and 8. A drug substance is considered highly soluble when the dose/solubility volume of solution are less than or equal to 250 ml. In addition, if the extent of drug absorption is greater than 90% given that the drug is stable in the gastrointestinal environment; it will be considered as a high permeable drug. With perhaps only few exceptions sink conditions are required to obtain in vitro dissolution curves representing the biopharmaceutical properties of the drug product under investigation with minimal effects due to the influence of solubility.

The purpose of in vitro dissolution studies in drug development process is to assess the lot-to-lot

quality of a drug product, guide development of new formulations; and ensure continuing product quality and performance after certain changes, such as changes in the formulation, the manufacturing process, the site of manufacture, and the scale-up of the manufacturing process. However, from the IVIVC standpoint, dissolution serves as a surrogate for drug bioavailability. Thus more rigorous dissolution standards may be necessary for the in vivo waiver. Generally, a dissolution methodology, which is able to discriminate between the study formulations with different release patterns and best, reflects the in vivo behaviour should be used to establish an IVIVC. The in vitro dissolution release of a formulation can be modified to facilitate the correlation development. Changing dissolution testing conditions such as the stirring speed, choice of apparatus, pH of the medium, and temperature may alter the dissolution profile. 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.

Four basic types of dissolution apparatus including rotating basket (Apparatus 1), paddle method (Apparatus 2), reciprocating cylinder (Apparatus 3) and flow through cell (Apparatus 4) are specified by the USP and recommended in the FDA guidance. However the first two methods are preferred and it is recommended to start with the basket or paddle method prior to using the others unless shown unsatisfactory. Reciprocating cylinder has been found to be especially for bead type modified-release dosage forms. Apparatus 4 may offer advantages for modified release dosage forms that contain active ingredients with very limited solubility. Apparatus 5 (paddle over disk) and apparatus 6 (cylinder) have been shown to be useful for evaluating and testing transdermal dosage forms.

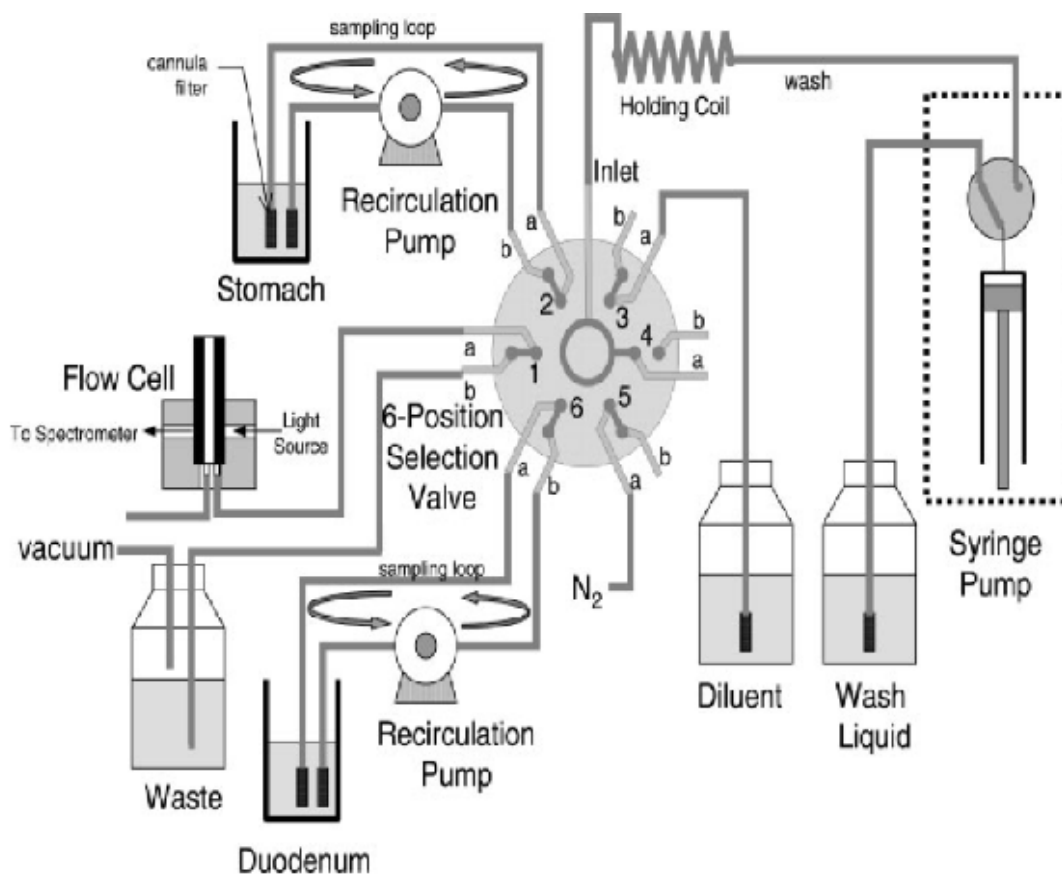
In general an aqueous test medium is preferred. The pH of dissolution medium, however, differs slightly between various guidance. Water which is allowed by some guidance's or buffered solution preferably not exceeding pH 6.8 is recommended by FDA as the initial medium for development of an IVIVC. As recommended by USP, deaerated water, a buffered solution (typically pH 4 to 8) or a dilute acid

(0.001 to 0.1 N) may preferably be used as dissolution medium for modified-release dosage forms. To simulate intestinal fluid or gastric fluid a dissolution medium of pH 6.8 or pH 1.2 should be employed respectively. Since the drug solubility depends on the composition of the

dissolution medium, surfactants, pH, and buffer capacity play a major role in drug solubility in the GI tract. For poorly soluble drugs, therefore, addition of surfactant (e.g., 1% sodium lauryl sulphate) may be appropriate. In general, non-aqueous and hydro-alcoholic systems are discouraged unless supported by a documented IVIVC. More extreme testing conditions (e.g. pH>8) should be justified. Strict simulation of physiologic gastrointestinal environment is not recommended and addition of enzyme, salts and surfactants need to be justified.

1. We have reported<sup>1</sup> the development of an artificial stomach-duodenum (ASD) apparatus (Fig.3) for the study of in vitro dissolution of solid active pharmaceutical ingredients (APIs) and formulations. Unlike a USP dissolution apparatus, the ASD consists of two chambers representing the stomach and the duodenum portions of the gastrointestinal (GI) tract. As in the in vivo system, the fluids in the upper chamber flows down towards the lower chamber and this transport produces more representative dynamic conditions in the ASD. In the ASD model, it is assumed that the concentration of the drug in the simulated duodenum is proportional to its bioavailability, i.e., the amount of drug absorbed is proportional to the amount present in solution in the simulated duodenum chamber. The importance of considering fluid and particle transport in the experimental parameters is critical in improving in vivo–in vitro correlation. In a dynamic setup such as the ASD, the fluid transport and infusion of fresh simulated GI fluids causes a continuous variation in the sink condition in the chambers. This condition is more reflective of the in vivo conditions and may offer more favorable results in predicting in vitro performance compared to other in vitro methods.

One of the problems in the initial ASD design was the detection system, which used UV–Vis fiber optic probes directly inserted in each chamber. In cases where high concentrations of particulates were present, the suspended solids scatter and block the light in the probe. This caused abnormally high background intensity in the spectrum that generally made it impossible to quantify the concentration. We have modified the ASD to include an internal sampling and dilution system to overcome such issues and also allow the analysis of solid dosage forms containing high concentration of drug substance and insoluble excipients.



**Fig.9. Schematic diagram of the internal sampling system in the artificial stomach-duodenum (ASD) apparatus**

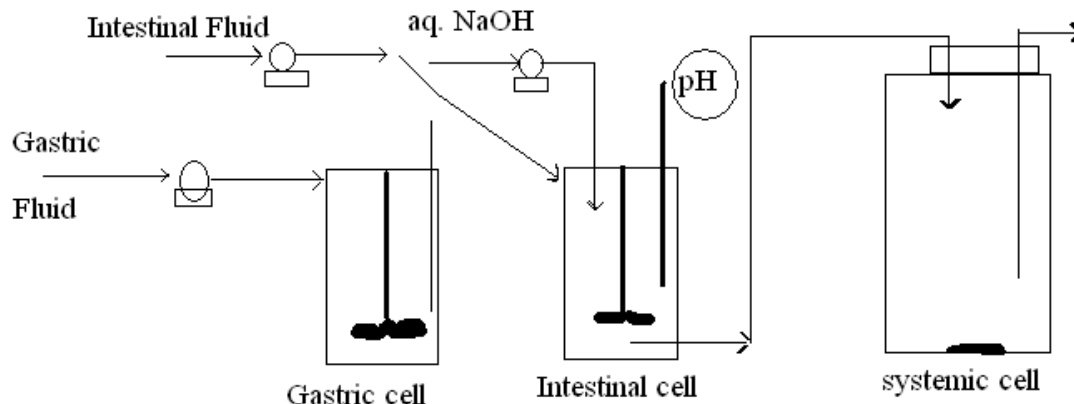
## 2. TNO intestinal model (TIM1) <sup>(22-31)</sup>

Nutrition and food research (Netherlands). Simulating GI track in man, this is artificial digestive system (ADS). Can maintain possibility of introducing a solid meal to investigate all food-drug interaction and food impact on the drug dosage form and also used in nutrition studies.

ADS method is a multi compartmental dynamic, computer controlled system that simulates human GI track was used. TIM1 is composed of four serial compartments simulating the stomach, the duodenum, the jejunum and the ileum. All parameters of system were adjusted to simulate condition found in GI tract in a healthy adult in fasted and fed state.

The new ADS method was developed by Dr. L. Hughes and A. Gehris (Rohm and Haas Research Laboratories - Spring House). This method involves equipment which is shown schematically in fig.4. This comprises 3 continuous, stirred cells connected in series. Simulated gastric fluid is pumped into first cell (gastric cell). The effluent from gastric cell is fed into second cell

(intestinal cell) together with simulated intestinal fluid and some NaOH (to neutralize acid). Effluent from the intestinal cell is fed into third cell (systemic cell). All three cells are operated at constant volume. The dosage form to be tested is added to first cell where it disintegrates and starts to dissolve. As soon as particles get small enough they will start to transfer to intestinal cell, just as small particles will start to leave the stomach in vivo.



**Fig. 10 Artificial Digestive System**

The fluid transferring from gastric cell to systemic cell will therefore be a mixture of drug dissolved in gastric fluid and undissolved drug and excipient. During this transfer the fluid is mixed with simulated intestinal fluid. In the intestinal cell the pH is controlled at typical intestinal pH, and undissolved drug will continue to dissolve. Note that undissolved drug cannot leave this cell, so that the overall effect is that dissolved drug is removed. This is equivalent to intestinal absorption, the rate of removal will be first order in drug concentration – a very good approximation of the in vivo absorption kinetics. Finally the dissolved drug enters the systemic cell. In this cell the kinetics of removal will be first order, & can be considered equivalent to clearance from blood plasma. The test method has been evaluated using some drugs viz. Ibuprofen, Pseudoephedrine, Paracetamol and Diclofenac. These drugs represent wide range of absorption, clearance and solubility.

There are some limitations to this method that need to be identified

- **Absorption rate:** Anything that changes absorption rate constant will not be assessed by this method, eg. Absorption



- **First pass metabolism:** The method does not address First pass metabolism. However, if the First pass metabolism is linearly dose related and not saturated, then the correction is a linear factor and correlation will be unaffected.
- **First order clearance:** First order clearance kinetics is implicit in method. Major deviations from this assumption will not be assessed by this method.

## CONCLUSION

IVIVC includes in vivo relevance to in vitro dissolution specifications and can serve as surrogate for in vivo bioavailability and to support biowaivers. Furthermore, IVIVC can also allow setting and validating of more meaningful dissolution methods and specifications. It can also assist in quality control for certain scale-up and post-approval changes. Both the regulatory agencies and pharmaceutical industries have, therefore, understood this value of IVIVCs. The FDA Guidance on IVIVC provides general methods is considered as an important complimentary tool for use in in vitro dissolution techniques, allowing more information to be collected about drug behaviour in various GI conditions & in which all drug of high dosage can be easily administered. The ADS method is sensitive, effective & reliable in predicting in vivo performance of dosage forms. The number of studies reported in the area of establishing IVIVCs for non-oral dosage forms are very scarce and further research is necessary in the development of more meaningful dissolution and permeation methods.

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