Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research

ISSN No: 0975-7384
CODEN(USA): JCPRC5

J. Chem. Pharm. Res., 2011, 3(5):550-564

In vitro–In vivo Correlation: Application in pharmaceutical development of various dosages forms

Bankim C Nandy[1*], Sandipan Roy[2], Bhaskar Mazumder[3], Kailash C Meena[1], Dharmedra Ahuja[1], Manju Makhija[1], Swati Jain[1], Stuti Sharma[1], Arti Shrivastava, Priya Saxena[1]

[1]Department of Pharmaceutical Sciences, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India
[2]Dr. Reddy's Laboratories Limited, Formulation R&D, Innovation Plaza, Hyderabad, India
[3] Department of Pharmaceutical Sciences, Dibrugarh University, Assam, India

ABSTRACT

Recently 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. A good correlation is a tool for predicting in vivo results based on in vitro data. IVIVC allows dosage form optimization with the fewest possible trials in man, fixes dissolution acceptance criteria, and can be used as a surrogate for further bioequivalence studies; it is also recommended by regulatory authorities. Most correlations between in vitro and in vivo data (IVIVC) rely on linear relationships. However, nonlinear IVIVC can be also observed, justified and validated. Thus the need for a tool to reliably correlate in vitro and in vivo drug release data has exceedingly increased. Such a tool shortens the drug development period, economizes the resources and leads to improved product quality. Increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development as the main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers. It supports and/or validates the use of dissolution methods and specification settings. This review article represents the FDA guidance, development, evaluation, and validation of an IVIVC to grant biowaivers, and to set dissolution specifications for oral dosage forms, biopharmaceutics classification systems (BCS), BCS biowaivers, application of BCS in IVIVC development and concept of mapping. The importance of dissolution media and methodology and pharmacokinetic studies in the context of IVIVC has been highlighted. The same principles of IVIVC used for oral extended release products may be applied for non-oral products such as parenteral depot formulations and novel drug delivery systems as well.

Key words: Correlation, Convolution, Deconvolution, Dissolution, BCS

INTRODUCTION

In vitro in vivo correlation (IVIVC) is established to enable a dissolution test to be used as a surrogate of the bioavailability study [1]. It is basically related the amount of drug dissolved *in vitro* to the amount of drug absorbed *in vivo* using appropriate mathematical functions and suitable dissolution test conditions. The *in vivo* drug performance then is predicted based on the correlation function as well as dissolution parameters. Many studies reported in the late '70s and early '80s established the basic concept of IVIVC [2].Various definitions of *in vitro–in vivo* correlation have been proposed by the International Pharmaceutical Federation (FIP),the USP working group [3],and regulatory authorities such as the FDA or EMEA [4–6]. The FDA [4] defines IVIVC as "a predictive mathematical model describing the relationship between an *in vitro* property of an extended release dosage form (usually the rate or extent of drug dissolution or release) and a relevant *in vivo* response, e.g., plasma drug concentration or amount of drug absorbed." As stressed in this definition, IVIVC is more an *in vitro–in vivo* relationship than a strict correlation.

Convolution and deconvolution are standard mathematical tools for the analysis of linear systems, based on the validity of the superposition principle. Application to IVIVC problems dates back to the 1960's, e.g. by work of Silverman and Burgen [7], Rescigno and Segre [8], and Hanano [9]. Around 1980, several authors elaborated the technique, e.g. Vaughan and Dennis [10], Cutler [11-14], Veng-Pedersen [15-18], and the present author [19-23]. In several papers [24-28] it was stressed the relationship with pharmaco-dynamic response. The technique as such became 'official' by guidelines of USP [29] and FDA [30].According to these, it can be performed on three levels ranging from most to least informative, and briefly summarized as follows:(A) convolution and deconvolution of entire time profiles; (B) moments as metrics based on convolution relationships; (C) empirical metrics such as Cmax or tmax. For the present state of art, the reader is referred to actual textbooks [31], monographs [32]; and relevant software packages [33, 34].

Although the focus of discussion, in this review, will primarily be centred on modified-release formulations for which IVIVC is believed to be more defined, various aspects of the IVIVC of immediate-release dosage forms are also discussed. This review article represents the FDA guidance, development, evaluation, and validation of an IVIVC to grant biowaivers, and to set dissolution specifications for oral dosage forms, biopharmaceutics classification systems (BCS), BCS biowaivers, application of BCS in IVIVC development and concept of mapping. The importance of dissolution media and methodology and pharmacokinetic studies in the context of IVIVC has been highlighted. The same principles of IVIVC used for oral extended release products may be applied for non-oral products such as parenteral depot formulations and novel drug delivery systems as well.

Purposes of IVIVC

The optimization of formulations may require changes in the composition, manufacturing process, equipment, and batch sizes and 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). The main purpose of an IVIVC model is to utilize *in vitro* dissolution profiles as a surrogate for *in vivo* bioequivalence and to support biowaivers and data analysis of IVIVC attracts attention from the pharmaceutical industry and also to predict the entire *in vivo* time course from the *in vitro* data [3, 4, 23, 32].

Various Steps to Design and Develop of IVIV Correlation

i) Develop formulations with different release rate such as slow, medium and fast or a single release rate if dissolution is condition independent.

ii) Obtain *in vitro* dissolution profiles and derive *in vitro* dissolution parameters to be correlated.

iii) Obtain *in vivo* plasma concentration profiles by definitive bioavailability studies of these formulations and estimate the *in vivo* absorption or dissolution time course by proper data treatment i.e.- by applying methods of residual or Wagner Nelson method or Loo-riegelman method.

iv) The *in vitro* dissolution is compared by three ways like single point, statistical moment correlation and Deconvolution and Convolution Correlation Technique. Simply positioning one curve over another-the in vitro dissolution curve and the *in vivo* input rate curve are either directly superimpossible or may be made by intercity factor [3, 4, 32-35] (I), (eq. 1).

$$\mathbf{I = t_{50\%} \text{ for absorption} / t_{50\%} \text{ for dissolution}} \qquad \mathbf{Eq.\ 1}$$

[$t_{50\%}$ means time require to absorb/ dissolve 50 % of the initial drugs]

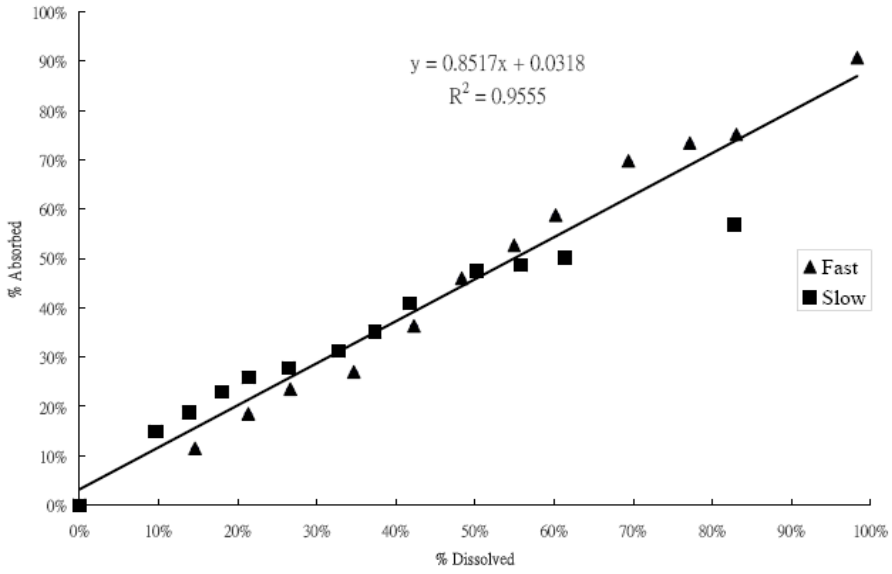

Fig. 1.The correlation between *in vitro* drug dissolution and *in vivo* drug absorption

This may also be quantified by defining the equation for each curve and comparing the corresponding constant such as slope (m) and intercept(c) by plotting the graph between fraction absorbed *in vivo* vs the fraction released by the *in vitro* studies.

Levels of IVIV Correlation

The concept of correlation is based on its ability to reflect the entire plasmatic concentration time curve, obtained after the administration of the dosage form. It is the relationship between the entire *in vitro* dissolution curves to the entire curve of plasmatic levels of drug which defines the correlation or correlation could be referred to as the relationship between appropriate *in vitro* release characteristics and *in vivo* bioavailability parameters [3, 4].

There are total five levels of correlation i.e. A, B, C, D, and multiple Level C, have been addressed in the IVIVC guidance.

Level A correlation is the highest level of correlation achievable as it is a point to point relationship between *in vitro* dissolution and the *in vivo* absorption rate of a drug from the dosage

form. A correlation of this type is generally linear and represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* input (eq. 2) rate (e.g., the *in vivo* dissolution of the drug from the dosage form). In a linear correlation, the *in vitro* dissolution and *in vivo* input curves may be directly super imposable or may be made to be super imposable by the use of a scaling factor. Nonlinear correlations, while uncommon, may also be appropriate. Alternative approaches to developing a Level A IVIVC are possible. Whatever the method used to establish a Level A IVIVC the model should predict the entire *in vivo* time course from the *in vitro* data. In this context, the model refers to the relationship between *in vitro* dissolution of an ER dosage form and an *in vivo* response such as plasma drug concentration or amount of drug absorbed. The predicted fraction of the drug absorbed is calculated from the observed fraction of the drug dissolved.

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 superimposable, 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 [36-38]

% *in vivo* input (t) =α+β [% *in vitro* input (t))] **Eq. 2**

[α and β are the intercept and slope of the regression line, respectively]

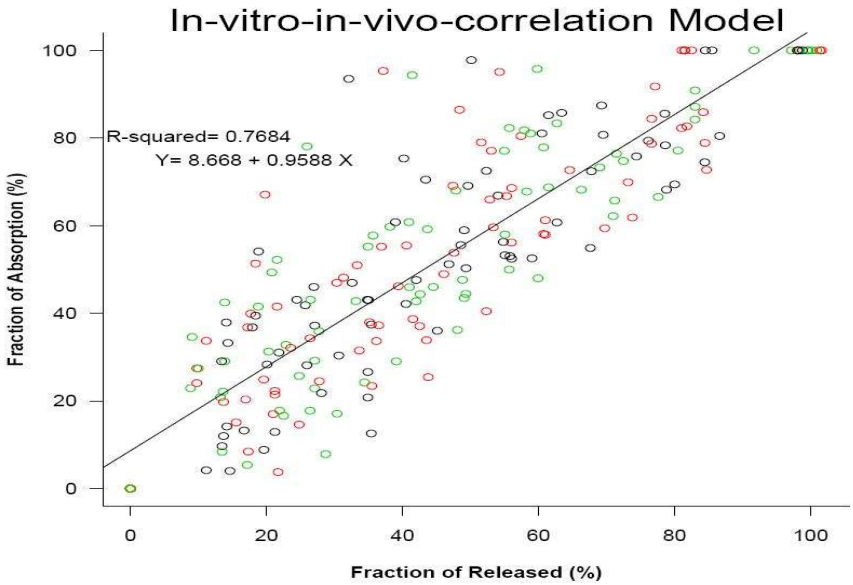

Fig. 2.The correlation between *in vitro fraction of* drug released and *in vivo fraction of* drug absorbed

Level B compares the mean *in vitro* dissolution time (MDT, eq. 4) to the mean. A Level B IVIVC uses the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared either to the mean residence time or to the mean *in vivo* dissolution time. A Level B correlation does not uniquely reflect the actual *in vivo* plasma level curve, because a number of different *in vivo* curves will produce similar mean residence time values. *In vivo* dissolution or residence time (MRT, eq. 3). These parameters can be determined by statistical moment theory (2, 3).

$$MRT = \frac{\int_0^{\infty} tc\ dt}{\int_0^{\infty} c\ dt} = \frac{AUMC}{AUC} \qquad \textbf{Eq. 3}$$

$$MDT = \frac{\int_0^{\infty} tD\ dt}{\int_0^{\infty} D\ dt} \qquad \textbf{Eq. 4}$$

[Where as, area under the first moment curve is AUMC or the area under the curve observed for the product of time and concentration versus time and area under the plasma concentration time curve (AUC) is one of the most basic parameters necessary for pharmacokinetic data analysis and is well used as a measure of drug disposition.]

Level C is a single point comparison of the amount of drug dissolved at one dissolution time point to one pharmacokinetic parameter. A Level C IVIVC establishes a single point relationship between a dissolution parameter, for example, $t_{50\%}$, percent dissolved in 4 hours and a pharmacokinetic parameter e.g., AUC, C_{max}, T_{max}). The methods and criteria for assessing the predictability of level C correlation are same as these of level A correlation [2, 3].

Limitation: This is a weak correlation since it does not reflect the plasma or dissolution profiles. A Level C correlation does not reflect the complete shape of the plasma concentration-time curve, which is the critical factor that defines the performance of ER products. In addition to these three levels, a combination of various levels C is also described and known as multiple level C [39-40].

Multiple Level C is a correlation involving one or several pharmacokinetic parameters to the amount of drug dissolved at various time points. Its correlation is more meaningful than that of Level C as several time points are considered. A multiple Level C correlation relates one or several pharmacokinetic parameters of interest to the amount of drug dissolved at several time points of the dissolution profile. 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. It should be based on at least three dissolution time points covering the early, middle and later stages of the dissolution profiles [37-39].

Level D is a rank order and qualitative analysis and is not useful for regulatory purpose.

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

Level	*In vitro*	*In vivo*
A	Dissolution curve	Input (absorption) curves
B	Statistical moments: MDT	Statistical moments:MRT, MAT,etc
C	Disintegration time, Time to have 10,50,90% dissolved, Dissolution rate, Dissolution efficiency	C_{max}, T_{max}, K_a, Time to have 10,50,90% absorbed, AUC (total or cumulative)

This study, however, focuses only on the development of the Level A, B, and C IVIVCs.

Applications [37, 41-44]

Level A correlations use all the information of the dissolution and absorption curves, in contrast to levels B or C. The establishment of a relationship implies the use of many formulations, each of them giving one pair of data (vitro and vivo). The FDA ranked the levels as follows: A Level A IVIVC is considered the most informative and is recommended, if possible.

Level B correlations are least useful for regulatory purposes. It is obvious that **level B or C** needs more data and, as they do not use all the information related to vitro and vivo behaviour of the formulation, they are less powerful.

Level C correlations does not allow prediction of the actual performance of the *in vivo* product, it can be useful in the early stages of formulation development when pilot formulations are being selected or as a production quality control routine or it is useful only as a guide to the development of formulations.

However, if a **multiple Level C** correlation is possible, and then a Level A correlation is also likely and is preferred. Multiple Level C correlations can be as useful as Level A correlations.

Level D is not a formal correlation but serves as an aid in the development of a formulation or processing procedure

In vivo data are obtained from well-standardized fasted studies on healthy volunteers. In attempting to establish a Level A relationship, the major point to consider is the sampling schedule in the "absorption" phase in order to have an accurate representation of the input curve. Establishing an IVIVC is nothing more complicated than trying to reproduce all the complex phenomena that lead to the *in vivo* release and solubilisation of the API in the gut in a "simple" *in vitro* system like a vessel agitated with a paddle. In contrast to *in vivo* studies, *in vitro* methods are less "standardized," as USP Apparatus I to IV could be used with various media (HCl, simple buffer, addition of surfactant or enzymes, etc.) and various technical parameters (e.g., volume, rate).

Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System (Amidon et al., 1995) classifies drugs into four categories (Table 1), depending on their solubility and permeability characteristics [35-40].

Table 2: The Biopharmaceutics Classification System (BCS)

Class I	Class II	Class III	Class IV
High Solubility	Low Solubility	High Solubility	Low Solubility
High Permeability	High Permeability	Low Permeability	Low Permeability

According to this scheme, Class I drugs should be more than 90% absorbed. Class II drugs are those with solubilities too low to be consistent with complete absorption, even though they are highly membrane permeable. Class III is the mirror image of Class II. These drugs have good solubility but are unable to penetrate the gut wall quickly enough for absorption to be complete. Class IV compounds have neither sufficient solubility nor permeability for absorption to be complete. Note, though, that although they certainly do not possess optimal properties, some drugs in this category may still be absorbed well enough to permit oral administration. Correlation of *in vivo* results with dissolution tests is likely to be best for Class II drugs, because in this case the dissolution rate is the primary limiting aspect to absorption. The other case where good *in vitro* / *in vivo* correlations (IVIVCs) are often obtained is when a Class I drug is formulated as an extended release product, since in this case; too, the release profile controls the rate of absorption [43-46].

Solubility criteria defined in present regulatory guidance for classifying an Active Pharmaceutical Ingredients (API) as "highly soluble" requires the highest strength to be soluble in 250ml of water over the pH range of 1-7.5 at 37^0C, otherwise it is considered as poorly soluble [41]. The FDA and also EMEA Guidance define "highly permeable" as having a fraction dose

absorbed of not less than 90%. The recently adopted WHO guidelines set a limit of not less than 85% of the fraction dose absorbed, otherwise it is considered to be poorly permeable.

Biowaiver for BCS Class I

On the basis of FDA guidelines, sponsor can request biowaiver for BCS Class I in immediate release solid oral dosage form, if the drug is stable in GIT and having narrow therapeutic index with no excipient interaction affecting absorption of drug in the oral cavity. Once a drug enters in stomach; it gets solubilised in gastric fluid rapidly before gastric emptying and the rate and extent of absorption is independent of drug dissolution as in case of solution. Hence, the goal of biowaiver is achieved [47].

Biowaiver Extension Potential for BCS Class II

The rate and extent of absorption of BCS Class II drug depends on *in vivo* dissolution behaviour of immediate release products. If *in vivo* dissolution can be predicted from *in vitro* dissolution studies, *in vivo* bioequivalence study can be waived. *In vitro* dissolution methods can mimic *in vivo* dissolution behaviour of BCS Class II drug and are appealing but experimental methods can be difficult to design and validate because of number of processes involved [45].

Biowaiver Extension for BCS Class III

If excipient used in two pharmaceutically equivalent solid oral immediate release product does not affect the drug absorption and the products dissolves very rapidly (>85% in 15 min.) in all relevant pH ranges, there is no reason to believe that these products would not be bioequivalent.

Approaches for Development of Correlation [5, 14-16]

Basically, there are at least three correlation techniques available in the pharmaceutical sciences. Single point, statistical moment and convolution and deconvolution techniques are discussed in terms of the advantages of each along with the resulting potential utility as a predictive tool for the user. Since both the deconvolution and convolution techniques and the statistical moment calculations utilize all of the dissolution plasma level data available to develop the correlations, they represent a major advantage over the single point approach.

Single Point Correlation Technique:

This technique represents the correlation between one dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to one pharmacokinetic parameter. It is generally only useful as a guide in formulation development or as a production quality control procedure. It does not reflect the complete shape of the plasma level, which is the critical factor that defines the performance of the dosage form. Thus, this correlation technique is not predictive of actual *in vivo* product performance [46, 48]. Level C correlation can be established by this technique, but because of its obvious limitations, it has limited usefulness in predicting IVIVC.

Smolen co-workers and other researchers [25-28] pointed out that since the selection of these single correlative points is usually arbitrary, the interpretation of the results can be misleading. More preferable would be the correlation of the entire *in vivo* response time profile to the complete dissolution rate time curve. Such correlation can only result in developing dissolution tests that predict reliably the time course of the *in vivo* behavior of the drug.

Statistical Moment Correlation Technique:

The concept of Mean Residence Time (MRT) based on statistical moments provides one method for correlating *in vivo*-*in vitro* data. The theory of statistical moments is based on the preliminary assumption that the movement of the individual drug molecules through the body compartment is governed by probability. Furthermore, the time course of drug concentrations in plasma can usually be regarded as a statistical distribution curve [4, 49]. Level B correlation is based on

correlating mean time parameters that characterize the *in vitro* and *in vivo* time courses. If a good correlation exits between the MRT for *in vitro* dissolution and MRT for a suitable *in vivo* disposition parameter, then the relatively simple procedure of monitoring the dissolution profile should allow the prediction of *in vivo* availability. By definition, MRT is the average time a drug molecule spends in the introduced kinetic space. It depends on the site of input and the site of elimination. The traditional area under the plasma concentration time curve (AUC) is one of the most basic parameters necessary for pharmacokinetic data analysis and is well used as a measure of drug disposition. MRT is the time when 63.2% of an intravenous dose has been eliminated. This concept is similar to the biologic half life, the time required for 50% of a dose to be eliminated. MRT may be calculated as the ratio of the area under the first moment curve (AUMC) to the AUC, where AUMC is the area under the curve observed for the product of time and concentration versus time. The true MRT of a drug in the body may be calculated only when the actual time course of the amount of drug in the body is known and is independent of the details of transport within the body [30, 50]. Many of the applications of statistical moment theory have stemmed from a desire to characterize drug absorption in a noncompartmental fashion. These methods are also applicable to nonparenteral routes of drug administration other than the oral route. Some researchers [31, 51] first proposed mean absorption time as a novel method to characterize the rate of drug absorption in bioavailability studies. Some researchers [32, 52] extended this theory by virtue of the additivity of various transit times, including the mean absorption time (MAT), which summarizes the mean time for drug molecules to remain unabsorbed.

$$MAT = MRTiv - MRTni \qquad \text{Eq. 5}$$

MAT is simply the difference in MRT following intravenous administration (MRTiv) and another noninstantaneous administration (MRTni). In the same manner, the mean dissolution time (MDT) of a solid dosage form may be determined by the difference in MAT for solid dosage form and a solution of the drug substance. There are some limitations to pharmacokinetic data treatment using statistical moment theory. These relationships become more complex when a distribution component or a two compartment model is necessary to describe the data, and elimination must be assumed to occur only from the central compartment. A rigorous experimental design must be used to provide sufficient sampling during the absorption phase and more importantly, during the terminal elimination phase. Recognition of these limitations may preclude any inaccuracies in determination of various pharmacokinetic parameters based on statistical moment theory. Among others, noncompartmental analysis methods based on statistical moment theory are becoming increasingly popular for rapid data analysis by investigators.

Deconvolution approach

This involve estimation of *in vivo* absorption profile from plasma drug concentration - time profile using Wagner Nelson or Loo-Riegelman method, subsequently the relationship with *in vitro* data is evaluated. Model dependent deconvolution methods are based on mass balance. The approximate equation used in absorption analysis for the two-compartment model was first published by Loo and Riegelman in 1968. Wagner published an exact Loo-Riegelman method for a multicompartment model in 1983. This is a general equation for absorption analysis of one- to three- compartment models [52-54].

In a deconvolution approach, hypothetical drug release profiles calculated by numerical deconvolution from the urinary excretion data obtained after per oral administration of the sustained release tablet formulation (as a response function) and reference solution (as a weighting function) were compared with drug release profiles obtained *in vitro* under various experimental conditions. The predicted and experimentally obtained drug release profiles *in vivo*

were correlated using linear regression analysis, proportional odds (Eq. 6), proportional hazards (Eq. 7) and proportional reverse hazards model (Eq. 8)

$$\frac{Fi2(t)}{1- Fi2\ (t)} = \alpha i \frac{Fi1(t)}{1- Fi1(t)} \quad \text{Eq. 6}$$

$$1 - Fi2(t) = (1 - Fi1(t))^{\alpha i} \quad \text{Eq. 7}$$

$$\text{Log}\ (Fi2(t)) = \alpha i \log (Fi1(t)) \quad \text{Eq. 8}$$

Where ¸αi corresponds to the constants of proportionality and Fi1 and Fi2 are the distribution functions for *in vitro* and *in vivo* dissolution times, respectively.

However, the rate profile obtained by this procedure is applicable only for the analysis of *in vivo* dissolution kinetics for the test sample. A deconvolution-based IVIVC model is typically established using a two-stage approach, i.e., deconvolution calculation to estimate the time course of *in vivo* absorption and/or release followed by comparison with *in vitro* fraction released. The IVIVC is assessed and validated by statistically comparing the predicted with the observed plasma levels. This convolution based modeling focuses on the ability to predict measured quantities rather than on indirectly estimated "*in vivo*" fraction absorbed and/or released. Thus, the results are more readily evaluated in terms of the effect of *in vitro* release on *in vivo* performances, e.g., AUC, Cmax and duration above minimum effective concentrations. Vaughan and Leach also utilized the numerical deconvolution method for absorption rate calculations and the prediction of plasma drug profiles from *in vitro* dissolution data. However, criticized the deconvolution mathematical predictive technique used by Smolen and other as too complicated and expensive because it requires special computers equipped with Fourier transform capabilities. Instead, a simpler method based on statistical moment theory can be recommended [55-58].

One stage convolution approach:

Convolution is a model independent method based on the superposition principle. It computes the *in vivo* absorption and simultaneously models the *in vitro – in vivo* data. In a convolution approach, cumulative urinary excretion profiles *in vivo* were predicted using drug release profiles obtained *in vitro* (as an input function) and mean cumulative urinary excretion data obtained for reference preparation (as weighting function). The predicted *in vivo* profiles were correlated with mean cumulative urinary excretion profiles observed *in vivo* using linear regression analysis [59].

Table 3: Development of Correlation

TWO STEP APPROACH	**Step 1:** Estimate the *in vivo* absorption or dissolution time course using an appropriate technique for each formulation and subjects. **Step 2:** Establish link model between *in vivo* Predict plasma concentrations from in *in vitro* using link model.
ONE STEP APPROACH	Predict plasma concentration from an *in vitro* profile using a link model whose parameters are fitted in one step i. Do not involve deconvolution ii. Link model is not determined separately iii. Can be done without reference like IV bolus.

Two stage methods allows for systematic model development while one stage obviates the need for administration of an intravenous, oral solution or IV bolus dose. Mostly IVIVC models developed are simple linear equation between *in vitro* drug released and *in vivo* drug absorbed.

But sometimes these data can be better fitted by using nonlinear models like Sigmoid, Weibull, Higuchi or Hixon-crowell [60].

Deconvolution versus Convolution Approach:

Deconvolution approach represents a valuable tool for the identification of drug products *in vivo* dissolution kinetics. However, convolution computations are needed in order to predict drug products *in vivo* behaviour based on its *in vitro* drug release data. Although both approaches may be used in the course of IVIVC development, convolution approach resulted in a higher level of correlation and was less sensitive to the differences in drug release kinetics obtained under various experimental conditions *in vitro* [60-63].

Dissolution Methodologies, Apparatus and Classification

To design and develop dissolution methodologies as well as to derive complementary statistical techniques for unbiased dissolution profile comparison, USP 27 & NF22 (11) now recognize seven dissolution apparatus specifically and describes with allowable modifications in detail. The choice of dissolution apparatus should be considered during the development of the dissolution methods, since it can affect the results and duration of the test. The type of dosage form under investigation is the primary consideration in apparatus selection. The compendial apparatus for dissolution as per USP are: Apparatus 1 (rotating basket), Apparatus 2 (paddle assembly), Apparatus 3 (reciprocating cylinder), Apparatus 4 (flow-through cell), Apparatus 5 (paddle over disk), Apparatus 6 (cylinder), and Apparatus 7 (reciprocating holder) [3, 64].

The European Pharmacopoeia has also adopted some of the apparatus designs described in the USP, with some minor modifications in the specifications. In the European Pharmacopoeia, official dissolution testing apparatus for special dosage forms (medicated chewing gum, transdermal patches) have also been incorporated. Table 4 shows the different dissolution apparatus [65-66].

Table 4: Apparatus classification in the European Pharmacopoeia for different dosage forms

Dosage Form	Apparatus
Solid Dosage Form	Paddle Apparatus, Basket Apparatus, Flow – Through Apparatus
Transdermal Patches	Disk Assembly Method, Cell Method, Rotating Cylinder Method
Special Dosage Forms Chewing Apparatus (Medicated Chewing Gums)	Flow-Through Apparatus

Dissolution Medium

The most important parameters which are considered for simulating *in vivo* conditions are pH, buffer composition, buffer capacity, temperature, volume, hydrodynamics etc. Four suitable media for simulating the composition of proximal GI tract are SGF plus surfactant (fasted state / stomach); long life milk, 3.5% fat (fed state /stomach); FaSSIF (fasted state / small intestine) and FeSSIF (fed state / small intestine) [67, 68] .

Typical surface tensions in the stomach in the fasted state are on the order of 35–45 mN/m [69]. In order to simulate these conditions, a suitable surfactant can be added to the medium. Milk has been proposed to mimic gastric conditions after meal intake [68] since the ratio of carbohydrate: protein: fat is similar to that of a typical Western FaSSIF, the medium used to represent fasted state in the proximal small intestine, contains a phosphate buffer to achieve a pH of 6.5 and a buffer capacity of 10 m Eq per.

Non-compendial media have shown better IVIVC as compared to Compendial media which is listed in the official monographs. Hence non-compendial media have been proved to have discriminating power and are widely used. Basically, pH increases from small intestine to large intestine (pH 6.7- 8) due to which dissolution testing of extended release drug product should be carried out throughout entire physiological pH range (6.7- 8). Ionic strength of dissolution media also plays a vital role in dissolution testing. Ions present in the food and food induced secretions in G.I.T causes changes in ionic strength of G.I. fluid. Buffer capacity has importance in dissolution testing of formulation that contains acidic or basic excipients.

***In vitro* Dissolution Profile Comparison**

Where applicable, the similarity factor f_2 (65, 66) was calculated to indicate similarity of dissolution profiles under different test conditions. The f_2 value was calculated as follows in eq. 9:

$$f_2 = 50 \log [(1 + \frac{1}{n} \sum_{t=1}^{n} (Rt - Tt) \quad \text{Eq. 9}$$

Where log is logarithm base 10, n is the number of sampling points, Σ is the summation over all time points, and Rt and Tt are the cumulative percentage dissolved at each of the selected time points of the reference and test product, respectively. When the two profiles are identical, f_2 = 100. An f_2 value greater than 50 was the criterion for similarity between two dissolution profiles.

IVIVC of controlled release formulations

Controlled release formulations are linked to non-linear IVIVC

One would expect that all of these non-linear IVIVC studies correspond to immediate release formulations of sparingly soluble compounds. Surprisingly, this is not true. A significant proportion of non-linear IVIVC studies concern controlled release formulations [44], although these are supposed to be engineered to perform well in that respect. This discrepancy is due to the dramatic differences of the *in vivo* conditions to any *in vitro* experiment, regardless of the efforts to simulate the former with the latter as realistically as possible. These differences are related to the properties of the gastric fluids, such as composition and pH. The distinction is especially notable, as these are spatially heterogeneous and are altered by the presence of food, the mechanical conditions imposed by the physiology, such as complex motility and hydrodynamic patterns, and also other factors like feedback mechanisms and the synergistic effects of the interplay of various factors. Since what is important *in vivo* is not the release itself but the entire absorption process, factors that may not influence the release rate but influence the final pharmacokinetic profile also contribute to the observed variability and the discrepancies between the *in vitro* and the anticipated *in vivo* performance of controlled release dosage forms [45].

Anomalous diffusion-fractal / fractional kinetics

In the majority of cases, the release device controls the molecular diffusion of the drug molecules in and/or surrounding the delivery system.

This category includes the following pre-programmed delivery systems:

(i) polymer membrane permeation-controlled drug delivery systems;
(ii) polymer matrix diffusion controlled drug delivery systems;
(iii) polymer (membrane/matrix) hybrid-type drug delivery systems; and
(iv) microservoir partition controlled drug delivery systems [46].

While under *in vitro* conditions, the performance of these devices is very reproducible and the variability observed is quite low, under *in vivo* conditions, under-stirring and the heterogeneous properties of the medium, change the topology of the environment and influence the diffusion in the matrix, altering the kinetics of drug release. Unlike the well stirred *in vitro* experimental conditions, where the concentration of the medium is considered homogeneous, under *in vivo* conditions, due to the composition of the medium, a depletion zone may be developed around the device that alters the kinetics inside the device as well. It has been shown that in constrained, under stirred spaces diffusion of materials follows different laws than the classic Fickian law. These give rise to the so-called anomalous kinetics to emphasise the deviation from the classic case [47]. Although anomalous kinetics have been used to describe the *in vitro* drug release inside the device [48], this type of kinetics may extend outside the device as well as in a space which is wrongly assumed to be well stirred. Anomalous kinetics has been described by employing concepts of fractal geometry to account for the fact that due to the prevailing conditions, the space appears as if it has geometry of lower dimensionality than the Euclidean space [49]. In fact, Monte Carlo simulations have been used to study drug release for Euclidean and fractal geometries and found that the Weibull model provides an adequate description of the release process [50–59]. Under *in vivo* conditions, the topological differences of the medium do not remain constant for the entire process of release and they vary in time and along the different parts of the GI lumen. They are also affected by the presence of food and mechanical influences, such as the intestine motility, since this act as stirring. Efforts to describe mathematically the anomalous kinetics include fractal kinetics with power law and time dependant rate coefficients [60]. Also more recently, attempts with differential equations of fractional order, the so-called fractional kinetics, have appeared [61] to describe anomalous kinetics, where the fractional order of differentiation is also related to the geometry of the space. It is interesting to note that the fractional version of a constant rate process gives rise to a power law solution, when integrated [62]. Therefore, it is plausible that a process which appears to have a constant rate under well stirred *in vitro* conditions, becomes anomalous (power law) under constrained *in vivo* conditions. This could be described in the context of fractional kinetics simply by changing the order of the derivative in the differential equation.

Mechanical–dynamical factors cause variability

Further to the alteration of the release kinetics *in vivo* and due to the differences of the properties of the medium, mechanical properties imposed by the physiology and the anatomy are important. Apart from intestinal motility and its contribution to stirring of the GI contents, the hydrodynamic properties such as the flow, which determines the residence time of the device and also its location in the GI lumen, may be important [63]. The presence of food delays flow and alters the entire transit profile. This is important, especially for drugs with site dependant absorption, as the residence profile of the device is altered which results in the drug being released in parts of the GI with limited absorption, including the stomach [64]. Although this altered transit profile may not influence the release pattern itself, it influences the pharmacokinetic profile and contributes to the observed variability and departure from the *in vitro* performance. In the same vein, gastric emptying can also have a role in the observed pharmacokinetic variability, especially since there is evidence of feedback mechanisms with some drugs [65] and also complex behaviour of the myoelectric complex controlling the function of the pylorus [66]. The latter has been also reported to exhibit chaotic behaviour [66].

Synergistic actions produce complex behaviour

It is common in a multi factorial system that the synergistic effects of its components may give rise to emergent behaviours that cannot be explained by the individual behaviours of the components. Studying these systems in the classic reductionist approach may result in missing some of its properties. It is the interactions of the different components that give rise to these additional properties of the system. There is evidence that the GI may present such properties

and some of its components may form a dynamical system [45] with complex behaviour as a result of the interaction of these factors. We already mentioned that there have been reports of chaotic behaviour of the myoelectric complex. Another potential example of such a synergistic behaviour consists of three tightly interacting quantities: the drug concentration, food and biliary secretion. It is well known that the consumption of a meal rich in fat, stimulates alterations in gastric pH, secretion of pancreatic enzymes, and promotion of lymphatic transport and stimulation of biliary lipid release from the gallbladder. The basic components of biliary lipids, namely, bile salts, phospholipids and cholesterol promote the formation of colloidal species within the small intestine, which aid the solubilization of the poorly-water soluble products of lipid digestion, e.g., fatty acids as well as enhance the solubility of the poorly-water soluble drugs. The exact structure and function of the dynamical system food/ biliary lipids/drug is unknown; however, a recent study [64-67, 70] demonstrates that even relatively low quantity (2 g) of long chain lipids stimulated gallbladder contraction and elevated, variable intestinal bile salt, phospholipids and cholesterol levels. Although the variability was mainly attributed to classical randomness (subject to subject variation) the second reason quoted on the difficulties associated with effective duodenal sampling is most likely linked with the heterogeneous spatial composition of the intestinal fluids. Besides, the oscillatory nature of the concentration–time plots of the biliary derived lipids in the intestinal lumen might be indicative of the dynamics of the system. For all above mentioned reasons we believe that the *in vitro* measurements of drug solubility in food mimicking media or bile salts (bio-relevant media) cannot capture the dynamics of the *in vivo* conditions. Due to the multiple interactions among the components of the system involved, a reductionist approach focusing exclusively on the drug/ biliary lipids interaction cannot unveil the entire picture. Consequently, more carefully designed *in vivo* studies are required to shed light on the function of food/ biliary lipids/drug dynamical system.

CONCLUSION

In conclusion, we believe that the variability of the GI can be explained at large by contribution from dynamical sources, such as feedback mechanisms and complex behaviour, resulting from the interaction of the different components. Also, the altered topological properties of the GI contents seem to be particularly important for controlled release dosage forms, as these properties change the release rate from the device. However, one thing is clear; there are still too many unknowns in the GI system. This is the main reason why the discrepancies between *in vitro* behaviour and the observed *in vivo* performance, together with the corresponding variability, are treated as random noise and little attempt is made to explain them systematically, in a mechanistic way. More research, with experimental studies with emphasis on *in vivo* techniques and also applications of new theory with novel mathematical approaches is needed, to shed light on the processes of *in vivo* release, diffusion, transit and absorption, so that the discrepancies with the well described *in vitro* experiments can be explained [69-72]. In parallel, investigators should be encouraged to submit for publication non-linearly correlated or uncorrelated *in vitro* and *in vivo* data. This will enable a better understanding of the factors involved in this exercise.

REFERENCES

[1] U.S. Department of Health and Human Services. Guidance for industrial. Extended release oral dosage forms: Development, evaluation and application of *In vitro*/*In vivo* correlations. Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Rockville, MD, **1997**.

[2] J Skelly; G Amidon; W Barr; L Benet; F Carter; J Robinson; V Shah; A Yacobi. *Pharm.Res*., **1990**, 7,975–982.

[3] *In vitro* and *In vivo* Evaluation of Dosage Forms . In *USP 26–NF 21*; United States Pharmacopeial Convention, Inc.: Rockville, MD, **2002**, 1088.
[4] In vitro Release Testing and In vivo Bioequivalence Documentation; Guidance for Industry; U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Drug Evaluation and Research (CDER),U.S. Government Printing Office: Washington, DC, **1997**.
[5] Note for Guidance on Development Pharmaceutics; European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit, Committee for Proprietary Medicinal Products, CPMP/QWP/155/96, London, U.K., **1998**.
[6] Note for Guidance on Modified Release Oral and Transdermal Dosage Forms: Section II (Pharmacokinetic and Clinical Evaluation); European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit, Committee for Proprietary Medicinal Products, CPMP/EWP/280/96, London, U.K., **1999**.
[7] M Silverman; SV Burgen. *J. Appl. Physiol.* ,**1961**,16, 911–913.
[8] A Rescigno; G Segre. *Drug and Tracer Kinetics*, Blaisdell Publishing Co, Waltham, MA, **1966**, 75.
[9] M Hanano. *Chem. Pharm. Bull*.,**1967**, 15, 994–1001.
[10] D.P. Vaughan, M. Dennis, Mathematical basis of point-area de-convolution method for determining *in vivo* input functions, J. Pharm. Sci. 67 (**1978**) 663–665.
[11] D.J. Cutler, Numerical de-convolution by least squares: use of prescribed input functions, J. Pharmacokin. Biopharm. 6 (**1978**) 227–241.
[12] D.J. Cutler, Use of polynomials to represent the input function, Ibid 6(**1978**) 243–263.
[13] DJ Cutler. *J. Pharmacokin. Biopharm*., **1978**, 6, 265–282.
[14] DJ Cutler. *Pharmacol. Ther*., **1981**, 14 ,123–160.
[15] P Veng-Pedersen. *J. Pharm. Sci*., 1980, 69 , 298–305.
[16] P Veng-Pedersen. *Ibid* ,**1980**, 69 , 305–312.
[17] P Veng-Pedersen. *J. Pharm. Sci*., **1980**, 69 , 312–318.
[18] P Veng-Pedersen. *J. Pharmacokin. Biopharm*., **1980**, 8 , 463–481.
[19] F Langenbucher. *Pharm. Ind*., **1982**, 44,1166–1172.
[20] F Langenbucher. *Pharm. Ind*., **1982**, 44, 1275–1278.
[21] F Langenbucher; H Moeller. *Pharm. Ind.*, **1983,** 45,623–628.
[22] F Langenbucher; H Moeller. *Ibid* , **1983**,45,629–633.
[23] F Langenbucher; J Mysicka. *Br. J. Clin. Pharmacol*.,**1985**, 19 ,51S–162S.
[24] VF Smolen; WA Weigand. *J. Pharm. Sci.*, **1976**, 65,1718–1724.
[25] VF Smolen. *J. Pharmakin. Biopharm*., **1976**, 4, 337–353.
[26] VF Smolen. *Ibid* , **1976**, 4, 355–375.
[27] VF Smolen; RJ Erb. *J. Pharm. Sci.*, **1977**, 66, 287–304.
[28] VF Smolen. Practical pharmacodynamic engineering in the design, development and evaluation of optimal drug products, in: HS Bean; AH Beckett; JE Carless. *Advances in Pharmaceutical Sciences*, **1982**, Academic Press, London, 145-161.
[29] USP23/NF18, k1088l. *In vitro* and *in vivo* evaluations of dosage forms, **1995**, 924–929.
[30] FDA. Guidance for Industry: extended release oral dosage forms: development, evaluation and application of *in vitro*/*in vivo* correlations, *Diss. Technol*., **1997**, 23–32.
[31] J Gabrielsson; D Weiner. *Pharmacokinetic and Pharmacodynamic Data Analysis: Concepts and Applications*, Swed. Pharm. Press, Stockholm, Sweden, **1997**, 275-291.
[32] D Young; JG Devane; J Butler. In vitro-In vivo Correlations, Plenum Press, New York, **1997**, 53-56.
[33] S Blanquet; E Zeijdner; E Beyssac; JP Meunier; S Denis; R Havenaar; M Alric. *Pharm. Res.*, **2004**, 21, 37–49.
[34] MN Martinez; GL Amidon. *J. Clin. Pharmacol*., **2002**, 42, 620–643.
[35] E Mathiowitz. *Encyclopaedia of Controlled Drug Delivery*. John Wiley & Sons., **1999**, 1, 425-434.

[36] J Dressman; J Krammer. Pharmaceutical Dissolution Testing. *Taylor & Francis Group*., **2005**, 1-34.
[37] G L Mattok; R D Hossie; I J McGilveray. *Can. J. Pharm. Sci.*, **1972**, 7, 84.
[38] TRD Shaw; K Raymond; M R Howard; J Hamer. *Br. Med. J*.,**1973**, 4, 763.
[39] LF Chasseaud; T Taylor; RM Major; IW Taylor; V Luckow; A Darragh; RF Lambe. *Arzneim- Forsch.*, **1983**, 33(2), 1298-1301.
[40] GL Amidon; H Lennerna¨s; VP Shah; JR Crison. *Pharm. Res*., **1995**, 12, 413–420.
[41] JB Dressman; D Fleisher. *J. Pharm. Sci.*, **1986**.75, 109–116.
[42] C Mircioiu ; I Mircioiu ; V Voicu ; D Miron. *Basic Clin. Pharmacol. Toxicol*., **2005,** 96 (3),262–264.
[43] JE Polli; JR Crison; GL Amidon. *J. Pharm. Sci.*, **1996**, 85 (7),753–760.
[44] S Hayes; A Dunne; T Smart; J Davis. *J.Pharm. Sci.*, **2004**, 93 (3), 571–581.
[45] P Macheras; A Iliadis. Modeling in biopharmaceutics, pharmacokinetics and pharmacodynamics: homogeneous and heterogeneous approaches, *Springer*, New York, **2006**, 200-206.
[46] JC Boylan; J Swarbrick. *Encyclopedia of Pharmaceutical Technology*, vol I, 2nd ed. Marcel Dekker, New York,**2002**, pp 811–852.
[47] BJ West; W Deering. *Phys. Rep.*, **1994**, 246, 1–100.
[48] L Ritger; NA Peppas. *J. Control. Release*, **1987**, 23–37.
[49] R Kopelman. *Science*, **1988**, 241, 1620–1626.
[50] K Kosmidis; P Macheras. *Int. J. Pharmaceut*., **2007**, 343, 166–172.
[51] K Kosmidis; P Argyrakis; P Macheras. *Pharm. Res*., **2003**,20.
[52] K Kosmidis; P Argyrakis; P Macheras. *J. Chem. Phys*., **2003**, 119, 6373–6377.
[53] A Bunde; S Havlin; R Nossal; HE Stanley; GH Weiss. *J. Chem. Phys*., **1985**, 83 5909–5913.
[54] A Barat; HJ Ruskin; M Crane. *Simul. Model. Pract.* ,**2006**, 14,843–856.
[55] A Barat; HJ Ruskin; M Crane. *Simul. Model. Pract.*, **2006**,14,857–873.
[56] R Villalobos; AM Vidales; S Cordero; D Quintanar; A Domnguez. *J. Sol–Gel Sci. Technol*., **2006**, 37, 195–199.
[57] R Villalobos; A Ganem; S Cordero; AM Vidales; A Domnguez. *Drug Dev. Ind. Pharm*., **2005**, 31, 535–543.
[58] N Haddish-Berhane; C Nyquist; K Haghighi; C Corvalan; A Keshavarzian; O Campanella; J Rickus; A Farhadi. *J. Control.Release*, **2006**, 110,314–322.
[59] V Papadopoulou; K Kosmidis; M Vlachou; P Macheras. *Int. J. Pharm*., **2006**, 309, 44–50.
[60] IM Sokolov; J Klafter; A Blumen. *Phys. Today*, **2002**, 55 (11) 48–55.
[61] W Weitschies; RS Wedemeyer; O Kosch; K Fach; S Nagel; E Söderlind; L Trahms; B Abrahamsson; H Mönnikes. J. Control. Release, **2005**, 108 (2–3), 375–385.
[62] PH Marathe; EP Sandefer; GE Kollia; DS Greene; RH Barbhaiya; RA Lipper; RC Page; WJ Doll; UY Ryo; GA Digenis. *J. Pharmacokinet. Biopharm.*, **1998**, 26 (1), 1–20.
[63] ZS Wang; Z He; JD Chen. *IEEE Trans. Biomed. Eng.*, **2004**, 51 (8), 1401–1406.
[64] GA Kossena; WN Charman; CG Wilson, B O Mahony; B. Lindsay; J.M. Hempenstall; CL Davison; PJ Crowley; CJ Porter. *Pharm. Res.*, **2007**, 24 (11), 2084–2096.
[65] JW Moore; H H Flanner. *Pharm. Technol.*, **1996**, *20*, 64–74.
[66] VP Shah; Y Tsong; P Sathe; RL Williams; *Dissolution Technol.*, **1999**, 6 (3), 15.
[67] E Galia; E Nicolaides; D Ho¨rter;R Lo¨benberg; C Reppas; JB Dressman. *Pharm. Res.*, **1998**, 15, 698-705.
[68] P E Macheras; M Koupparis; E Apostolleli. *Int. J. Pharm.*, **1987**, 36, 125–136.
[69] P Finholt ; S Solvang; *J. Pharm. Sci.*, **1968**, 57, 1322–1326.
[70] E Demrturk; L Öner. *J. Pharm. Sci*., **2003**, 28, 215-224.
[71] I Iran. *J Pharm Pharmaceut Sci* **., 2006**, 9 (2), 169-189.
[72] B S Tanwer, R Choudhary, R Vijayvergia, *J. Chem. Pharm. Res.*, **2010**, 2(2): 489-495