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A Systematic Approach to Develop Level A, *In-vitro* and *In-vivo* Correlation (IVIVC)-Ketoprofen BCS Class II Drug Example

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Abstract. Three modified releases; slow release (SR), moderate release (MR) and fast release (FR) matrix tablets of ketoprofen, BCS Class II drug, were manufactured by wet granulation method. The similarity factor (f2) was used to analyze dissolution data. Randomized, three way, crossover bioavailability study of 12 h was conducted on nine (9) healthy volunteers. The *in-vitro* release profile of ketoprofen in phosphate buffer pH 7.5 with SLS (sodium lauryl sulphate 1%) at 100 rpm was found most fit to develop level A IVIVC. A linear correlation (R2 > 0.9) was found in level A, C and multiple C but a non-linear relationship was observed in level B.

Keywords: IVIVC, matrix system, ketoprofen, correlation, similarity factor

Introduction

Bioavailability of a solid dosage form of a drug is influenced by the dissolution behavior under physiological conditions indicating *in-vitro* dissolution as pertinent for prophecy of expected *in-vivo* response of a drug product. Dissolution behavior depends upon release mechanism of drug product either its immediate release or an extended release formulation, and on the solubility and permeability as well. *In-vitro* dissolution testing is ascertained as a monitoring tool for evaluating the performance of a drug product, and it is also used as routine test for development and quality control of drugs and medicinal products. In development stage of a new drug product dissolution specifications are commonly established that are used as a yardstick for quality control of the drug product. Usually, the dissolution specifications are constructed from the range of dissolution values found in the lot used in the pivotal (*in-vivo*) bioavailability study, or from the range of values found from different lots produced during the development phase. Based upon those specifications, dissolution testing can then be used for assessing the lot to lot quality of a drug product, for ensuring a continuous drug product quality and performance after certain minor postapproval manufacturing changes (e.g. minor changes in formulation, or in the scale-up of the manufacturing process), and for guiding the development of new formulations of the drug substance. *In-vitro* dissolution testing usually must be performed as complementary to the *in-vivo* bioavailability investigations (Freitag, 2001; EMEA, 1999).

Amidon and co-workers made a major step in the theoretical analysis of oral drug absorption when solubility and dose were taken into account for the estimation of the absorption potential of a drug by using a pseudoequilibrium model (Amidon *et al*., 1995). Macheras and Symillides (1989) enabled the estimation of the fraction of dose absorbed as a function of absorption potential. However, the microscopic model based on mass balance considerations considered as a milestone in the history of oral drug absorption since it concluded that three fundamental dimensionless parameters dissolution, absorption and dose numbers govern extent of oral drug absorption (Oh *et al*., 1993). In fact these were used to describe dissolution of drug particles and influx of the dissolved drug. Based on this experimental work a nomenclature system called biopharmaceutical classification system (BCS), was established which categorize the medicinal moieties on the basis of their solubility, permeability and dissolution (Amidon *et al*., 1995). According to BCS four drug classes were defined *i.e*., high solubility/high permeability (Class I), low solubility/high permeability (Class II), high solubility/low permeability (Class III) and low solubility/low permeability (Class IV). The properties of drug substance were combined with the dissolution characteristics of the drug product, and predictions with regard to the *in-vitro–in-vivo* correlations (IVIVCs) for each of the drug classes were

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pointed out. These advances attracted the obvious interest of scientists in the importance of dissolution tests as predictors of oral absorption for Class II drugs (Dokoumetzidis and Macheras, 2006).

The BCS enabled the pharma scientists to predict relationship between *in-vitro* inputs and *in-vivo* outcomes that led to use *in-vitro* dissolution tests as a surrogate for *in-vivo* bioequivalence. In 2000, the FDA introduced regulatory guidance for BCS biowaivers. The BCS biowaiver guidance includes detailed instructions for classification of drugs according to the BCS as well as requisites for waiving the bioequivalence studies in the case of major product changes or in the development of new generic immediate-release drug products. IVIVC and BCS biowaiver guidelines have a remarkable potential for reducing the number of *in-vivo* bioequivalence studies.

The selection of dissolution method and the utilization of *in-vivo* data are critical steps in the development of a level A IVIVC model. The dissolution method should have acceptable discrimination ability, i.e., formulations with statistically significant differences in pharmacokinetic parameters should have dissimilar dissolution profiles. The *in-vitro* and *in-vivo* relationship is typically determined using averaged *in-vitro* and *in-vivo* data (Kortejärvi, 2008).

Correlation levels. Five correlation levels have been defined in the (IVIVC) FDA guidance. Levels of correlation depict the ability of a correlation to determine complete plasma drug level-time profile following administration of given dosage form (Emami, 2006).

Level A correlation. This is the highest category of correlation and represents a point-to-point relationship between *in-vitro* dissolution rate and *in-vivo* response of drug from the dosage form. 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. In level A correlation dissolution rate alone is sufficient to determine bioavailability of the product.

Level B correlation. Statistical moment analysis is applied to determine level B IVIVC. 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*). A level B correlation does not reflect real picture of *in-vivo* plasma profile. Therefore, it is not likely to 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.

Level C correlation. A level C correlation establishes a single point relationship between a dissolution parameter (e.g. $t_{50\%}$ or percent dissolved in 4 h) and a pharmacokinetic parameter (e.g. AUC or C_{max}). A level C correlation does not reflect the complete shape of the plasma concentration time curve, therefore is not the most useful correlation from a regulatory point of view. However, this type of correlation can be useful in early formulation development.

Multiple level C correlation. 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. Multiple level C correlation can be as useful as level A IVIVC from a regulatory point of view. However, if one can develop a multiple level C correlation, it is likely that a level A correlation can be developed as well.

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

In-vitro and *in-vivo* correlation (IVIVC) models are developed to explore the relationships between *in-vitro* dissolution/release and *in-vivo* absorption profiles. This model relationship facilitates the rational development and evaluation of immediate/extended-release dosage forms as a tool for formulation screening, in setting dissolution specifications and as a surrogate for bioequivalence testing. IVIVC modeling involves three stages, which are 1) model development, 2) model validation, and 3) model application to different scenarios. The principles of IVIVC model development have been successfully applied to oral dosage forms. However, the ground rules for developing and validating IVIVC models for novel and non-oral dosage forms/delivery systems (microspheres, implants, liposomes etc.) are still under investigation (Sunkara and Chilukuri, 2003).

It is clear from the above discussion that, pharmaceutical companies are eager for the rapid drug development

and approval, while regulatory agencies need assurance of the product quality and performances. During the last 25 years, there has been a considerable interest within the pharmaceutical industry, academia, and regulatory sectors *in-vivo* and *in-vitro* correlation of oral dosage form. In 1971, Wagner stated that future research in dissolution rates should be directed mainly towards establishing correlation between *in-vitro* and *in-vivo* data (Ghosh and Choudhury, 2009). The BCS Class II drugs exhibit low solubility and high permeability characteristics. The scientific rationale for granting biowaiver extension for Class II drugs is that their oral absorption is most likely limited by *in vivo* dissolution. If *in-vivo* dissolution can be estimated *in-vitro*, it is possible to establish an *in-vitro* and *in-vivo* correlation.

BCS II drugs have not been accepted as biowaiver candidates by the regulatory agencies, but acidic BCS II drugs have been suggested as possible candidates for biowaivers in scientific publications (Yasir *et al*., 2010a).

BCS Class II drugs exhibit low solubility and high permeability characteristics. The scientific rationale for granting biowaiver extension for Class II drugs is that their oral absorption is most likely limited by *in-vivo* dissolution. If *in-vivo* dissolution can be estimated *in vitro*, it is possible to establish an *in-vitro* and *in-vivo* correlation (Yasir *et al*., 2010b). The following areas require more extensive research:

1. Increase the dose volume for solubility classification to 500 mL.
2. Include bile salt in the solubility measurement.
3. Use the intrinsic dissolution method for solubility classification.
4. Define an intermediate solubility class for BCS Class II drugs.
5. Include surfactants in *in-vitro* dissolution testing, (Yu *et al*., 2002).

Level A correlations have a clearly defined regulatory benefit to the industry; lower level correlations (B and C) do not. As a result, the utility of level B and C correlations generally is limited to establishing guidance for early formulation development, or providing a qualitative or, at most, a semiquantitative method for evaluating formulation changes or stability changes during later phase development (Brown, 2004). The use of *in-vitro* methodology as a surrogate for *in-vivo* BE studies involves little therapeutic risk (Potthast *et al*., 2005).

In certain cases, especially for ER formulations, the dissolution test can serve not only as a quality control for the manufacturing process but also as an indicator of how the formulation will perform *in-vivo.* Thus, the main objective of developing and evaluating an IVIVC is to establish the dissolution test as a surrogate for human bioequivalence studies. The best dissolution method for *in-vivo* and *in-vitro* correlation is, obviously, the method that describes what happens *in-vivo*. The main factors that can influence drug release *in-vivo* are pH, surfactant, bile, movement, ionic strength, enzymes, food etc. All of these factors cannot be easily reproduced *in-vitro* by a simple dissolution method. In addition, the relative importance of these factors is not similar if the subject is in fed or fasted state; in the fed state, food also has a direct influence on the API or formulation behavior (Cardot *et al*., 2007).

Solinis *et al*. (2002) studied the release of salbutamol and ketoprofen enantiomers from hydroxypropylmethyl-cellulose (HPMC) K100M matrices containing two types of cellulose derivatives. The authors concluded that stereoselectivity is dependent on the amount of chiral excipient in the formulation (Gohel *et al*., 2005).

Corrigan *et al*. (2003) made a major contribution for development of IVIVC for the ketoprofen drug. The purpose of this work was to investigate the influence of dissolution medium composition on the *in-vitro* release of ketoprofen from a series of extended release (ER) products and the impact of the different buffer media on the *in-vivo* and *in-vitro* (IVIV) relationship. The products investigated were coated micro bead preparations having increasing levels of coating to retard drug release. Four common dissolution media; USP phosphate buffers of pH 7.2 and 6.8, phosphate (modified isotonic) buffer pH 6.8 and a fasted state simulated intestinal fluid without lipid components (FaSSIFLF) of pH 6.5, were employed in the USP 2 apparatus. The only apparent IVIVC incorporating all four ER products, which was non-linear, was obtained using the phosphate (modified isotonic) buffer of pH 6.8.

The *in-vitro* release of ketoprofen from proprietary and extemporaneously manufactured gels was observed in detail by Tettey and Amlalo (2005).

By taking the stimulus from the above mentioned work of (Tettey-Amlalo (2005), Corrigan *et al*. (2003) and Yu *et al*. (2002) it was planned to advance their work to achieve linear *in-vitro* and *in-vivo* relationship instead of non-linear relationship for the ketoprofen drug and

led to provide an easy and systematic approach to develop level A IVIVC for the pharmaceutical industries particularly of our own country.

Materials and Methods

As ketoprofen belongs to BCS Class II (low solubility/ high permeability) drug, it is considered a good candidate to develop expected IVIVC models. The process of developing IVIVC models was categorized into two stages:

1. Model development and validation
2. Model application

Model development and validation. Model development stage has been divided into following steps:

I. In accordance with FDA Guidance for Industry (1997), three modified extended release formulations of drug (ketoprofen = 200 mg) were developed using wet granulation technique by the Industrial Pharmacy Laboratory in the Department of Pharmacy,The Islamia University of Bahawalpur, Pakistan. Hydrophilic matrix approach was applied to manufacture the tablets using varying amount of excipients. HPMC K15 M and filler were used as retardant to control release of drug from matrix system. Finally tablets were coated with aqueous based film of opadry red. Blending time, drying time and compression force were kept constant. Three formulations were designed so as to have different release rates for release of ketoprofen referred to as Slow, Moderate and Fast. Dissolution behavior of formulations F1, F2 and F3 was determined by using Dissolution Apparatus USP II with agitation speed of 50 and 100 rpm. Aqueous media with pH range 1.2-7.5 were selected for dissolution (USP, 2009; Emami, 2006; Sunkara and Chilukuri, 2003; FDA, 1997).

II. Dissolution conditions were maintained at 37 ± 1 ºC. Dissolution samples were collected at zero, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 and 12 h. The amount of ketoprofen was quantified by UV-Spectrophotometer at 260 nm. Dissolution Vs time profile was recorded for each formulation under different dissolution conditions.

III. A single dose, crossover, randomized study pharmacokinetic study was conducted on nine (9) healthy volunteers. Following ethical standard protocol, experimental work was accomplished in the Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur, Pakistan. Subjects were aged between 18-40 years and free from any clinically significant abnormality on the basis of medical history, physical examination and laboratory evaluation comprising hematology, clinical chemistry, urinalysis, ECG, virology (hepatitis B and C, HIV) and drug screen (drugs of abuse and addiction) reports. Other exclusion criteria included history of gastritis, peptic ulcer, asthma, hypersensitivity to ketoprofen or alcohol abuse and deviation of more than 10% from ideal body weight for height. Subjects were fasted overnight (for at least 10 h prior to dosing) and remained fasted for 4 h post dosing, after which lunch was served. Randomized administration of each formulation i.e. F1, F2 and F3 was accompanied by 240 mL water. Health assessment including vital signs, physical examination and clinical laboratory testing was performed seven days before and after study. Subjects were interviewed at the beginning and end of each study period and monitored throughout the confinement period to determine any adverse events potentially related to study medication. The plasma sampling time points following administration of the test formulations, with a wash out period of 1 week, were: 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 & 12 h including zero time sampling. Blood samples were collected in heparinized plastic centrifuge tubes. Plasma was obtained by centrifugation at 3000 rpm for 10 min and immediately frozen at -60 °C (Ghosh *et al*., 2008; WHO, 2006).

IV. A validated HPLC method for the analysis of ketoprofen in human plasma was invented. The quantification of ketoprofen and I.S (diclofenac potassium) was conducted using a C18 hypersil ODS column with the mobile phase, 50% acetonitrile in water (v/v) containing ammonium acetate and triethylamine (TEA) set at flow rate 1 mL /min. The calibration curve and linearity was determined over the concentration range of 0.25 µg/mL to 40 µg/mL which were linear with R^2 = 0.9991 and 0.9982, respectively. The accuracy was found more than 81.32%. The absolute average difference of 0.18 between the observed concentrations for intra and inter day studies proved the stability of the sample over one month (Sarfraz *et al*., 2011).

In-vitro data obtained from each formulation (F1, F2 and F3) was interpreted by determining similarity factor "f_2" using Eq. No. 1. (Ghosh *et al*., 2008).

$$f_2 = 50 \log \left\{ \left[1 + -\frac{1}{n} \sum_{t=1}^{n} w\,(R - T)^2 \right]^{-0.5} \times 100 \right\} \quad \text{..... 1}$$

R and T are the percent dissolved at each time point for reference and test product, respectively. W is the optional weight factor.

V. Measured plasma concentrations were used to determine different pharmacokinetic parameters for each formulation (SR, MR and FR) by Kinetica version 4.4 software as given in Table 1.

VI. Fraction of drug absorbed (FDA) was calculated in all the volunteers for each formulation (SR, MR and FR). As the pharmacokinetics of ketoprofen followed one-compartment model, Wagner-Nelson method was used to determine the FDA at all time points (Hassanzadeh and Beckett, 1997). Following eq. no. 2 was used to determine FDA by Wagner-Nelson method using Kinetica Version 4.4 software.

$$Fa_t = \frac{(c_p + k\,[AUC]_{o\text{-}t})}{(k[AUC]_{o\text{-}\infty})} \quad \text{..... 2}$$

Fa_t = fraction of drug absorbed at time t
Cp = plasma drug concentration at time t
$[AUC]_{0\text{-}t}$ = area under the concentration time curve from time 0 to t
$[AUC]_{0\text{-}\infty}$ = area under the concentration time curve from time 0 to infinity
k = elimination rate constant.

A level A correlation was established between fraction drug absorbed (FDA) and fraction drug released (FDR) for each formulation separately. This plot provides basic information of the relationship (i.e. linear, nonlinear) between two variables. FDR was plotted on X-axis while FDA was plotted on the Y-axis (Sirisuth and Natalie, 2002).

VIII. In level B correlation, mean value of MRT for all formulations (F1, F2 and F3) was calculated by using Kinetica version 4.4 software and mean dissolution time (MDT) was calculated by the eq. no. 3, as given below:

$$MDT = (n/n+1)*k^{-1/n} \quad \text{..... 3}$$

k = is the release kinetic constant obtained from the best fit curve of the release behavior of the drug.

n = is the release exponent that was determined by Korsmeyer Peppas kinetic eq. no. 4, as given in Table 2.

$$F = K_{kp}tn \quad \text{..... 4}$$

F = is fraction drug released at time t, K_{kp} is release constant.

IX. Pooled FDR vs. pooled FDA (IVIVC) development usually requires two or more different releasing formulations in the model Thus, a combination of two or three formulations, such as S/M/F, S/F, M/F and S/M was used to develop ketoprofen IVIVC model. The data from those variables were pooled together and plotted on the same graph. The linear or non-linear regression on the pooled FDR and FDA was then performed. The regression line should be significant ($p<0.05$) and the slopes should not be significantly different from 1 (if linear model) (Ghosh *et al*., 2008).

Applications. After the successful level A correlation has been developed, biowaivers and dissolution specifications can be set for formulations falling within the release rate ranges of the IVIVC, provided that those formulations possess the same release mechanism.

Results and Discussion

***In-vitro* data analysis.** As per guidelines of FDA for IVIVC (Guidance for Industry, FDA, 1997), three modified release formulations with different release rates were developed for establishment of level A IVIVC. Although the percentage concentration of HPMC in formulations F1 and F2 was same but different diluents were having different effects on tablet properties especially on release rates. The increased release rate of F2 as compared to F1 formulation was due to water soluble lactose as compared to water insoluble dibasic calcium phosphate (Vuebaa *et al*., 2004; Bravo *et al*., 2002). The deceased concentration of HPMC *i.e*. 2% in F3 as well as change in ratio of diluents was the reason for fast release rate in comparison of F1 and F2. Hence, the formulations were designated F1 as slow

Table 1. Pharmacokinetic parameters for formulations (SR, MR and FR)

Formulation	C_{max} (µg/mL)	$AUC_{0\text{-}12\ h}$ (hr*µg/mL)	$AUC_{0\text{-}\infty}$ (hr*µg/mL)	$t_{1/2}$ (h)	MRT (h)	k (h^{-1})	T_{max} (h)
				Mean ± SEM			
SR	2.61 ± 0.06	18.56 ± 0.37	19.21 ± 0.36	1.64 ± 0.03	5.36 ± 0.07	0.43 ± 0.01	4.00 ± 0.00
MR	3.28 ± 0.08	22.97 ± 0.54	23.59 ± 0.54	1.53 ± 0.03	5.10 ± 0.04	0.46 ± 0.01	3.33 ± 0.24
FR	3.84 ± 0.03	26.11 ± 0.34	26.59 ± 0.27	1.32 ± 0.03	4.76 ± 0.02	0.53 ± 0.01	3.11 ± 0.11

release formulation, F2 as moderate release formulation and F3 as fast release formulation. Phosphate buffer (PB) with pH 6.8 and 7.5 was the most discriminating media for formulations F1, F2 and F3 in which the release of drug from formulations was comparatively distinguishable. Maximum release of drug from all developed formulations F1, F2 and F3 was seen in PB pH 7.5 with SLS at 100 rpm dissolution condition as shown in Fig. 1. The release rate of ketoprofen from formulation F1, F2 & F3 was as follow:

Table 2. Release rate constants for different release kinetics of three formulations

Formulation	Kosmeyer peppas (n)	Zero-order (k)	1st-order (k)	Higuchi (k)
SR	0.7342 ± 0.0262	7.737 ± 0.6940	0.2562 ± 0.073	29.51 ± 1.375
MR	0.7431 ± 0.0446	8.375 ± 0.9876	0.2573 ± 0.078	32.55 ± 1.892
FR	0.4778 ± 0.0439	7.105 ± 1.411	0.2106 ± 0.088	29.63 ± 2.748

F3 (FAST) > F2 (MODERATE) > F1 (SLOW)

The values of similarity factor, f_2 were calculated for all formulations fast release (FR), moderate release (MR) and slow release (SR) in each media. The observed f_2 values for each pair of formulations in PB pH 7.5 with SLS at 100 rpm were as given in Table 3.

***In-vivo* data analysis.** A 12 h pharmacokinetic study was conducted. Plasma sampling was followed with the same time points as for dissolution sampling. Mean plasma ketoprofen concentration vs. time profiles after each formulation are presented in Fig. 2.

Table 3. Similarity factor (f_2) for extended release formulations (SR, MR and FR)

Time (h)	PB pH 7.5 SLS-100		
	FS	MS	FM
3	31.13	70.04	37.68
5	27.78	58.40	37.41
8	29.03	53.16	39.82

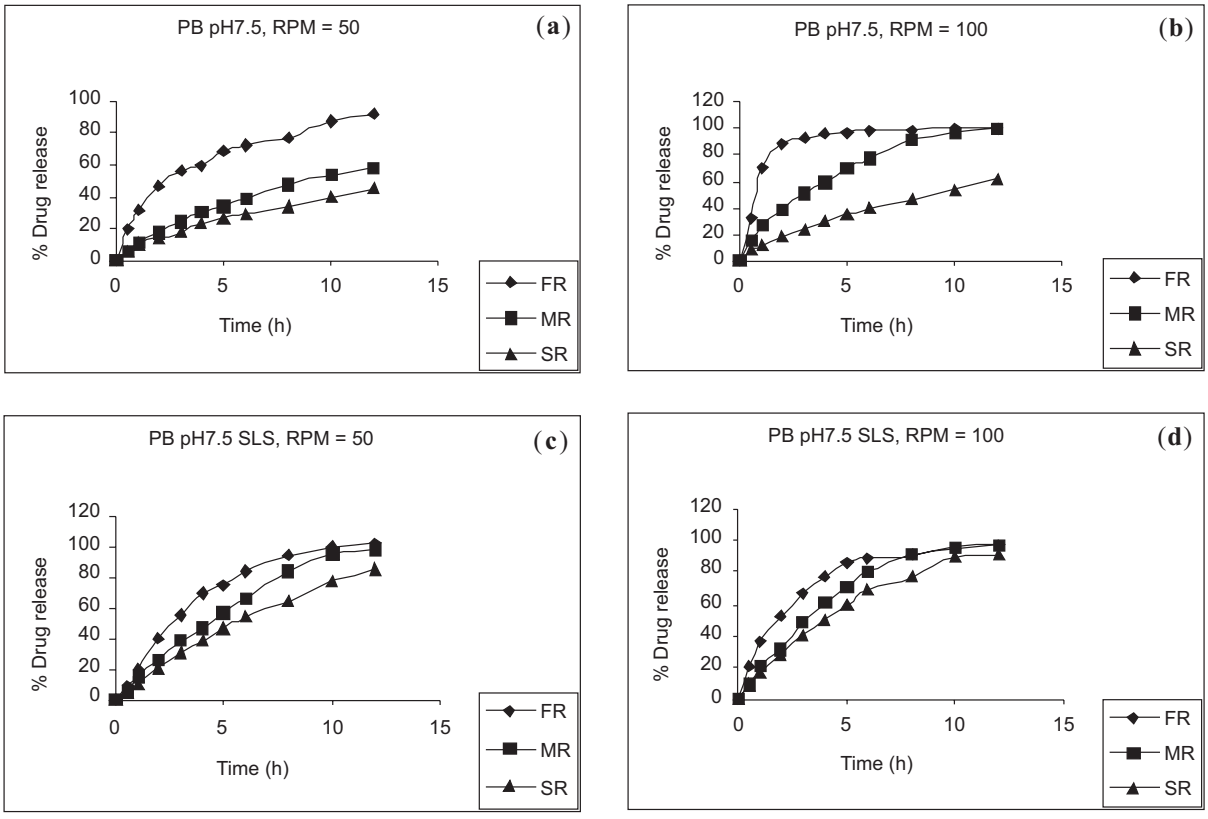

Fig. 1. Cumulative ketoprofen % release vs. time profile for slow, moderate and fast extended release tablets using phosphate buffer with pH 7.5 at (a) 50 rpm, (b) 100 rpm, (c) 50 rpm with SLS, (d) 100 rpm with SLS.

The mean pharmacokinetic parameters are summarized in Table 1. There was a discernible difference between plasma concentrations of each formulation. It was also observed that the rank order of release observed in the dissolution testing was also apparent in plasma ketoprofen concentration profiles with C_{max} 2.61, 3.16 and 3.82 for formulations SR, MR and FR, respectively. Similar case was observed with $AUC_{0\text{-}12\,h}$, $AUC_{0\text{-}\infty}$ and k. where as T_{max}, $t_{1/2}$ and MRT were found in decreasing order for formulations SR, MR and FR, respectively. It was observed that there was no significant difference between the values of k for each formulation showing that the rate of absorption of drug was controlled by matrix system. Higher value of k (> 0.4) showed that the metabolism of the drug was high in the body that may be due to fast acetylator volunteers. Low value of MRT also indicate fast metabolism of the drug. Higher value of k and lower MRT depicted the lesser $AUC_{0\text{-}\infty}$.

***In-vitro* and *In-vivo* correlation data analysis.** Level A correlation was investigated among mean FDR and mean FDA for all developed formulations (SR, MR and FR) using phosphate buffer pH 7.5 with SLS at 100 rpm. A good linear regression relationship was observed with R^2 values 0.9805, 0.975 and 0.9947 for SR, MR and FR, respectively as shown in Fig. 3. In accordance with FDA Guideline for Industry (2001), level A correlation data may be used as surrogate for bioavailability study. MDT is used to characterize the drug release rate from the dosage form and the retarding efficacy of the polymer. A higher MDT indicates a higher drug-retarding ability of the polymer and vice versa. The MDT value was found to be a function of polymer loading (Abdelkader *et al*., 2007). In level B, MRT values were plotted against MDT values. Non-linear (R^2 < 0.9) relationship was observed between MRT and MDT, but the values of MRT and MDT were seen to be in increasing order for the formulations FR, MR and SR, accordingly as shown in Fig. 4. Both the correlated parameters did not show high degree of correlation due to difference in their *in-vitro* and *in-vivo* kinetic behavior of each formulation i.e. there was a slow first order and first order kinetic module for the formulations F1, F2 and F3, respectively while their was same *in-vivo* kinetic module for each formulation (Razzak *et al*., 2008). Highest level of C correlation for all three formulations FR, MR and SR was observed at 6th hour (R^2 > 0.9) as shown in Fig. 5. At the start (first 2 h) of absorption and release profile, there was non-linear level C correlation. Level C correlation was a single time point relationship. The best correlation was

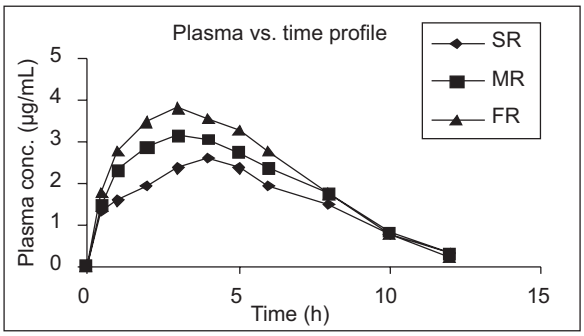

Fig. 2. Mean ketoprofen plasma vs. time profile for formulations (SR, MR and FR).

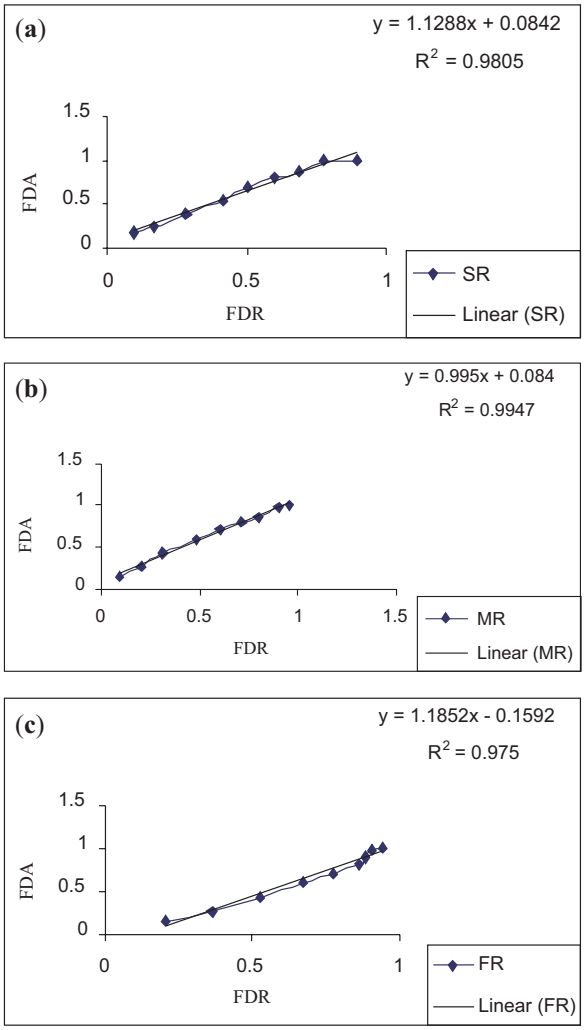

Fig. 3. Level A correlation between mean FDR vs mean (FDA) for formulation (a) SR, (b) MR and (c) FR

established at 6th h due to consecutive margin difference among % drug release for each formulation. Mean AUC_{cum} and % drug dissolved of each formulation were correlated at 2, 3, 4, 5 and 6 h time points to develop multiple level C correlation as shown in Fig. 6. A linear relationship (R^2> 0.9) of ketoprofen drug was observed at multiple level C. All the three formulations SR, MR and FR attained a same range of multiple level C. On the behalf of multiple level C correlation, the absorption of ketoprofen was formulation independent i.e. a zero order pharmacokinetics. It was concluded that dissolution was rate limiting step in the development of IVIVC of ketoprofen drug (Ette and Williams, 2007).

A linear relationship (R^2> 0.97) was found between pooled FDR and pooled FDA as shown in Fig. 7. The developed IVIVC models (S/M/F, S/M, M/F and S/F) may be used to predict expected *in-vivo* response for the developed formulations following future work.

The results of four models also showed point to point relationship among FDR and FDA values. The regression lines obtained between pooled FDR and pooled FDA for all IVIVC models were significant with p values < 0.001 and slopes were not significant from 1 (p values < 0.001) as given in Table 4.

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

Table 4. Regression parameters for IVIVC models of Ketoprofen matrix tablets

IVIVC model	Slope	Intercept	R	p value at 0.001
S/M/F	1.113	-0.00012	0.998	Significant
S/M	0.959	0.0520	0.999	Significant
M/F	1.096	-0.035	0.999	Significant
S/F	1.178	-0.047	0.997	Significant

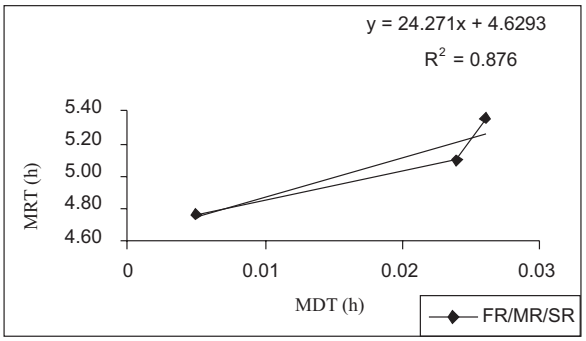

Fig. 4. Level B correlation between MDT vs. MRT.

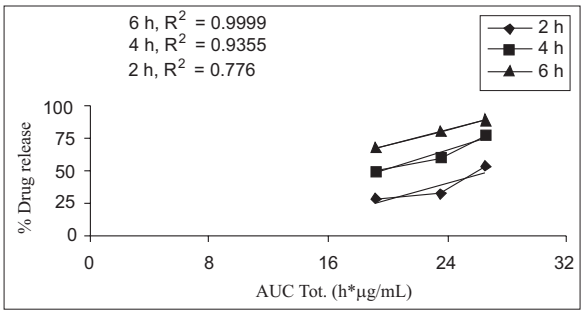

Fig. 5. Level C correlation between AUC Tot vs. % drug release

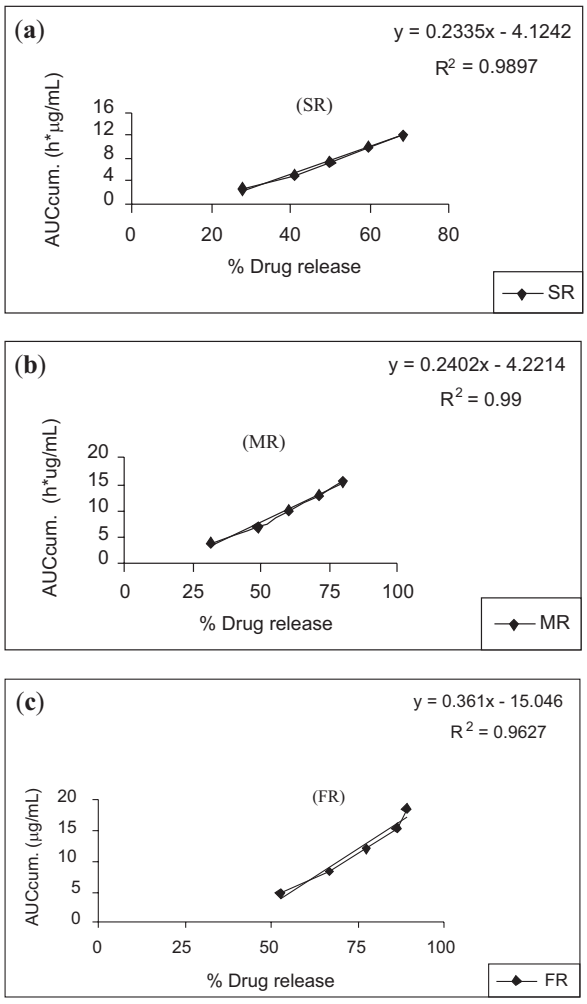

Fig. 6. Multiple level C correlation between AUC_{cum} vs. % drug release for (a) SR, (b) MR and (c) FR

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. It is an excellent quality control procedure since it is predictive of the dosage form's *in-vivo* performance (Yasir *et al*., 2010b). Achievement of level A correlation for ketoprofen represents its future prospect as biowaivers. A complete picture of the process to develop level A correlation for ketoprofen was illustrated in Fig. 8. The predicted ketoprofen plasma concentration was determined by the following procedure. First best fit kinetic model was obtained for the *in-vitro* data. The slope of the best fitting line was used as a rate of dissolution. The *in-vivo* dissolution rates were obtained from the *in-vitro* dissolution rates by using the different (S/M, S/F, M/F and S/M/F) IVIVC models. The prediction of the plasma ketoprofen was accomplished using the following curve fitting equation (Ghosh *et al*., 2003).

$$y = \text{const.} \times (\text{Dose}) \times K_a/K_a/-K_e \, (e^{-Ket} - e^{-Kat}) \quad \ldots\ldots 5$$

where:

y = predicted plasma conc, (µg/mL)

const. = F/Vd

where:

F = fraction of drug absorbed,
Vd = apparent vol. of distribution
K_a = absorption rate constant
K_e = terminal elimination rate constant

The de-convolution was accomplished on excel spread sheet.

The availability of a meaningful IVIVC of high quality and predictability for extended release formulation should provide a sound foundation for product development. Results of developed IVIVC models (S/M/F, S/M, M/F and S/F) proved the validity of the correlations that leads expected *in-vivo* outcomes. It also represents that what would be expected rate of absorption of the drug (Sirisuth and Natalie, 2001).

Level B correlations are least useful for regulatory purposes. Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected. Multiple level C correlations can be as useful as level A correlations.

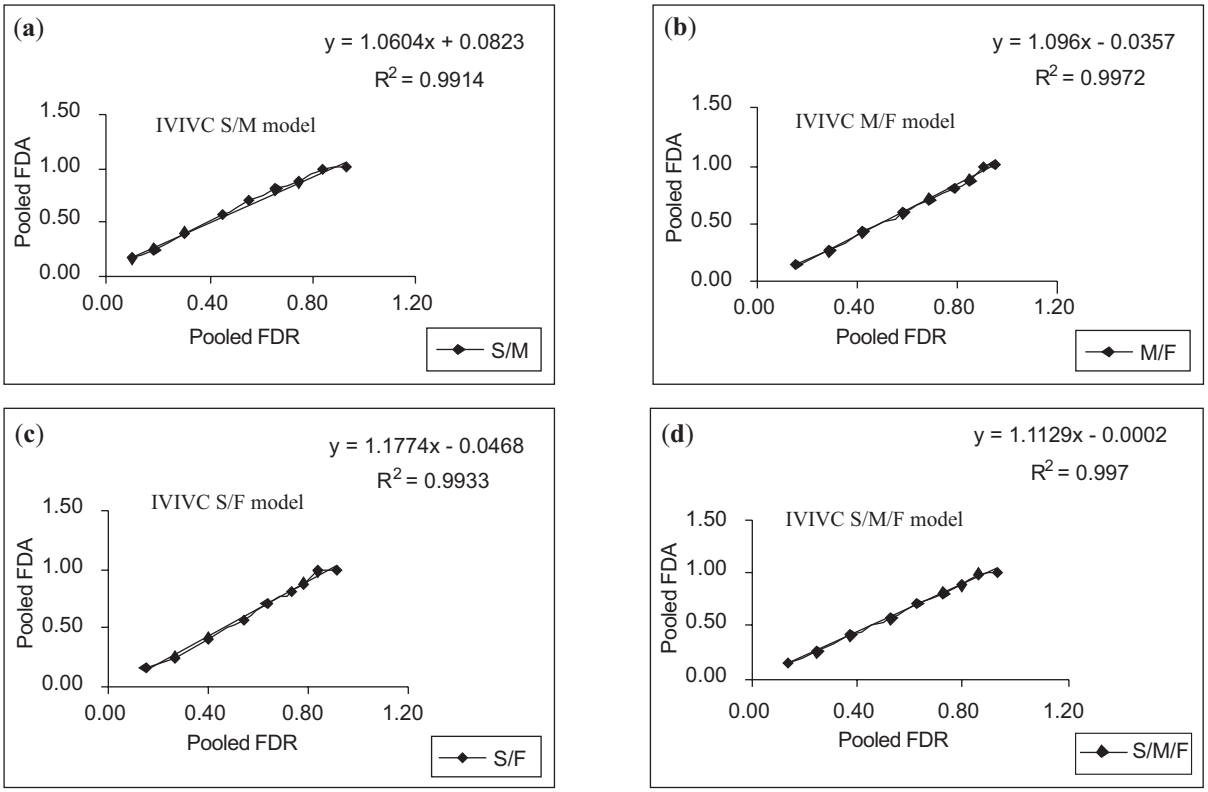

Fig. 7. IVIVC models (a) S/M, (b) M/F, (c) S/F and (d) S/M/F.

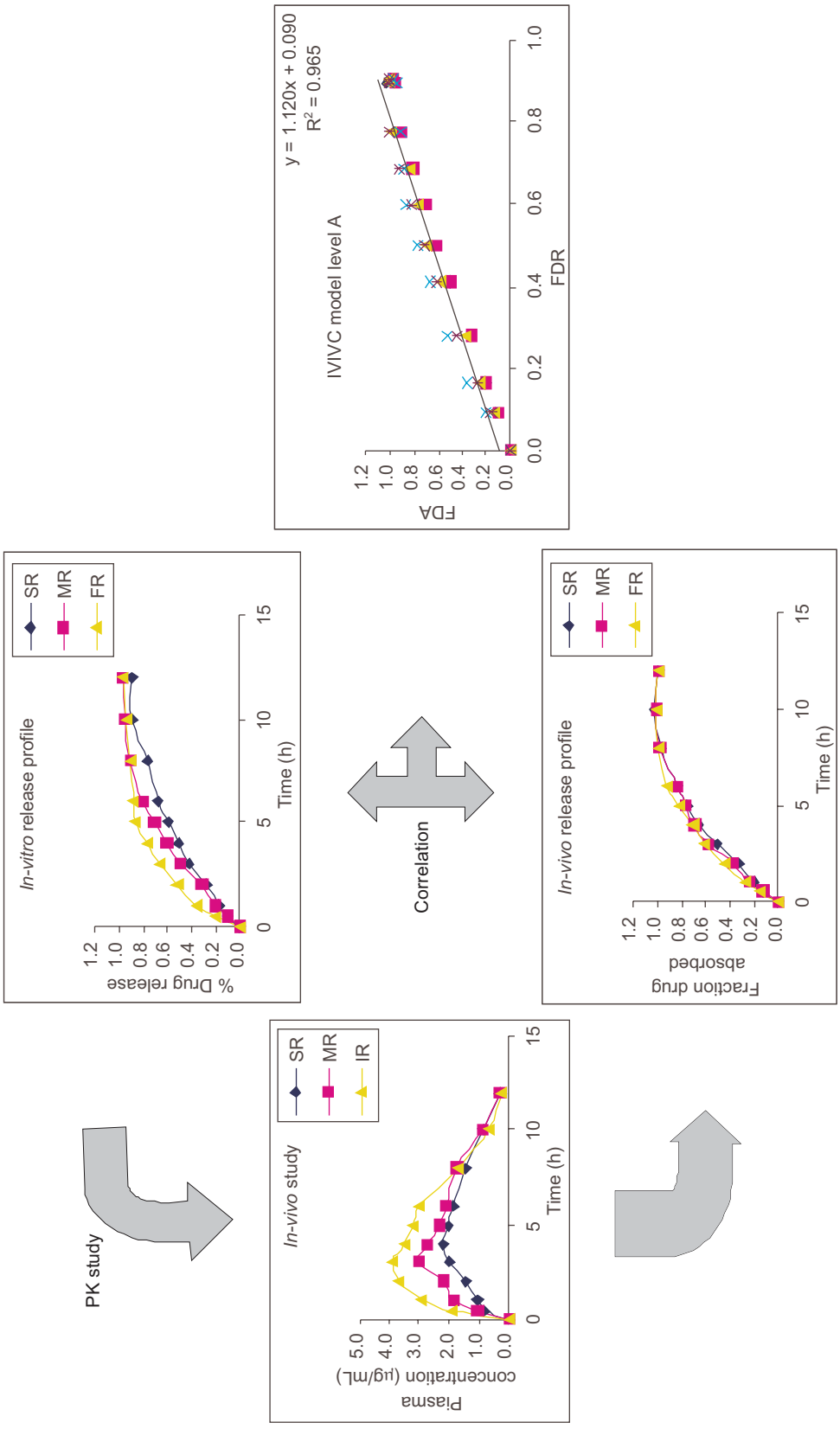

Fig. 8. Development of level A correlation.

However, if a multiple level C correlation is possible, then a level A correlation is also likely and is preferred (FDA, 1997).

Conclusion

The presented work was the continuation of the works of Tettey-Amlalo (2005); Corrigan (2003) and Yu *et al*. (2002). A linear level A IVIVC was established for the ketoprofen matrix tablets. An easy and systematic approach was adopted to achieve level A IVIVC that may be considered as a road-map particularly for the pharmaceutical industries to develop IVIVC model for the BCS Class II drugs.

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