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Research Article

Time Scaling for *In Vitro-In Vivo* Correlation: the Inverse Release Function (IRF) Approach

Jean Michel Cardot,[1,3] John C Lukas,[2] and Paula Muniz[2]

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Abstract. *In vitro-in vivo* correlations (IVIVC) are methods used to create a link between biopharmaceutical properties such as dissolution and physiological response such as plasma concentration. Level A IVIVC defines 1:1 relationship between the percent absorbed *in vivo* and the percent dissolved *in vitro*. A successful level A IVIVC provides the capacity to predict *in vivo* behavior based only on *in vitro* data with application in formulation development and support of biowaivers recognized by regulatory agencies across the world. Level A regression may be complicated due to differences in time scales as well as the lack of coincident times of similar release *in vitro* and *in vivo* leading to approximate time-to-time links and subsequent loss of information. Here, a novel method to establish Levy's plot and to provide time scaling for improved IVIVC predictive capacity is presented. The method is mathematically closed and is an inverse release function (IRF) characterizing the single (or more) phases of dissolution/absorption. It uses the complete set of information available from all time points both *in vitro* and *in vivo*. An extended-release formulation development situation is presented with three increasing release rate test products compared in a trial versus a reference product. First, the standard level A regression was made. Prediction errors for internal validation were higher than 10% for C_{max}. The IRF method was applied to obtain the *in vitro* times of percentage dissolved equivalent to percentage absorbed. The prediction errors from the IRF level A correlation were nearly negligible.

KEY WORDS: inverse release function; IVIVC; prediction; formulation design; time scaling

INTRODUCTION

In vitro-in vivo correlation (IVIVC) is a tool for optimizing formulation development by reducing the number of *in vivo* experiments or by limiting the risk of failure. Via a statistical regression, it links *in vitro* characteristics of the drug formulation with its corresponding pharmacokinetic (PK) response. IVIVCs are supported by regulatory authorities since the late 1990s and sponsors are encouraged to attempt establishment when developing extended-release formulations (1–4). IVIVC is crucial in quality by design approaches to verify the design space and establish critical and dissolution attributes (5,6). Dissolution methods can thus be optimized to be equally discriminative as the *in vivo* testing. A dissolution method with an established IVIVC can be used to assess differences in dissolution likely to have a relevant impact on *in vivo* absorption, facilitating fine tuning of test products towards successful pivotal trials. A successful correlation can assist in optimizing formulations via critical quality and manufacturing attributes, selection of appropriate dissolution acceptance criteria including widening dissolution specifications (depending on the predictive capacity of the IVIVC) and can be used as a surrogate for bioequivalence studies, as presented in different guidelines (1–4).

Often the highest level of IVIVC is first investigated, *i.e.*, the level A relationship. In classical two stage approaches, fraction dissolved, obtained from *in vitro* dissolution profiles is typically used together with corresponding *in vivo* fraction absorbed obtained by deconvolution of observed plasma concentrations, *e.g.*, after a comparative bioavailability trial. In this case, a predictive mathematical model is established between the *in vivo* absorption curve, considered to represent *in vivo* drug release and the *in vitro* dissolution curve through a 1:1 relationship between percent absorbed and percent dissolved.

However, utility of level A IVIVC is limited by differences between *in vitro* and *in vivo* processes. In spite of decades of collective experience in IVIVC establishment

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[1] Université Clermont Auvergne MEDIS, CHU Clermont-Ferrand, CIC1405, INSERM, 28, place H. Dunant-CS 60032, F-63000, Clermont–Ferrand, France.
[2] Dynakin SL, PTB 801, 48160, Derio, Vizcaya, Spain.
[3] To whom correspondence should be addressed. (e–mail: j-michel.cardot@uca.fr), URL: https://www.uca.fr

and application of regulatory guidelines, the majority of IVIVCs submitted to the FDA from 2008 to 2015 were not acceptable (6). A usual complexity in the establishment of correlations is the difference in time scale between *in vivo* and *in vitro* profiles. In fact, one cause of unsuccessful IVIVC reported by the FDA is incorrect use of *in vitro-in vivo* scaling factor among formulations (6). Discrepancies between *in vivo* and *in vitro* times are observed by faster *in vitro* dissolution compared to *in vivo* release or by differences in shape between the two curves. In both cases, a direct relationship between *in vitro* and *vivo* data cannot be set up simply.

The traditional tool to determine time scaling is the so-called Levy plot (7–9). Times at which *in vivo* and *in vitro* the same percentage is absorbed and dissolved, respectively, are plotted in the Levy plot. For time scale, *Tsc*, a regression of the form $t_{invitro} = Tsc \cdot t_{invivo}$ can possibly be established. The time scale is applied in the level A relation of fraction absorbed *vs*. fraction dissolved $F_{abs}(t_{invivo}) = F_{dis}(Tsc \cdot t_{invivo})$. Importantly, the time scale applied must be common for all formulations used to establish the IVIVC given the same discriminative dissolution method. The resulting relationship is different to direct plotting of percentage dissolved *vs*. percentage absorbed at the same time. This IVIVC after time scaling must be a linear 1:1 relationship, *i.e.*, slope equal to one and intercept to 0. The next step is to establish predictability relating the ability of the dissolution profiles to predict the typical *in vivo* bioequivalence parameters ($C_{\max}$, $AUC_{(0-t)}$) based on the established IVIVC. In practice, the above regression relationships are not always linear. Regression model development then could be subjectively driven by wrong hypothesis in order to, *e.g.*, account for different lag times, a change in the relationship after a certain percentage, or other challenges related to the data characteristics.

The aim of the present paper is to propose a closed mathematical method to achieve time scaling for IVIVC presenting the method via an example based on real data.

METHODS

In vivo and the corresponding *in vitro* data were obtained from a pilot trial (10) where three test extended-release (ER) formulations of increasing tablet size and corresponding dissolution speed (6 mm: fast, 7.5 mm: medium, 9 mm: slow) were compared to an immediate-release (IR) formulation.

The data was used to explore and establish level A correlations through direct regression and application of the inverse release function time scaling approach. The prediction errors obtained by both approaches were calculated. The terms "fraction" and "percentage" or "percent" are used when unit-less ratios of cumulative dissolution or deconvoluted cumulative ratio absorbed are expressed in scales of 0 to 1 or 0 to 100, respectively. Without loss of generality, the terms may be used interchangeably in the text.

All data manipulations, calculations, and graphics were performed using Phoenix WinNonlin® 8.1 (Certara Princeton, NJ, USA) and S-Plus® (TIBCO, Palo Alto, CA, USA) under MS Windows 10.

In Vivo

The example is based on pharmacokinetic observations from a pilot trial of a four-arm, single-dose, randomized cross-over design comparing three test ER tablets to the reference IR formulation from adult volunteers obtained in the scope of an IVIVC development. The treatment compound was an opioid abuse deterrent, extended-release tablet formulation of hydrocodone for once-daily dosing developed based on the novel proprietary Egalet® ADPREM technology. The trial and compound are described elsewhere (10). Briefly, the tablet is an injection molded polymer system consisting of an erodible matrix. The matrix is partly covered with a water-impermeable, non-erodible shell which leaves both ends of the cylindrical tablet exposed to erosion by the gastrointestinal (GI) fluid. The release of Egalet® ADPREM tablets is based on erosion rather than diffusion and is dependent on the location of the tablet in the gastrointestinal tract (11). The three ER formulations (fast: 6 mm width, medium: 7.5 mm width, and slow: 9 mm width formulations) were tested *in vivo versus* the IR formulations, NORCO®. For each formulation, *in vivo* PK were available in $N = 16$ subjects.

Validation of the IVIVC was done using the "all except one" method: two randomly selected ER formulations (7.5 and 9 mm) were used to establish the IVIVC and validate it by internal predictability and the third randomly selected tablet (6 mm) was used as an external predictability formulation.

A numerical deconvolution approach was used to derive the relevant cumulative fraction of drug dose absorbed percent (FDabs%) at time, *T*, from *in vivo* plasma concentration data using the IR formulation as UIR. The FDabs% refers to percentage of total amount absorbed (reaching systemic circulation) and is not fraction absorbed (Fa%) before any first pass. It is also different to fraction of dose absorbed in terms of absolute bioavailability; thus, FDabs% for IVIVC is only relevant for comparison of rates of absorption and not amount absorbed.

In Vitro

A dissolution method reflecting *in vitro* behavior for the three test compounds is available and based on a pharmacopeia paddle 50 rpm method leading to a constant erosion mechanism *in vitro* in contrast to *in vivo* that exhibits biphasic release of three formulations due to tablet location (10,11). Fractions dissolved percent (Fd%) were obtained for fast, medium, and slow formulations. Dissolution profiles were fit via a Weibull function as follows:

$$Fd(t) = Wb(t) = F_{inf} \cdot \left[1 - \exp\left(-\frac{t}{\mathrm{MDT}}\right)^{b}\right] \quad (1)$$

where F_{inf} is the maximum dissolved amount. MDT is the mean dissolution (or release) time, *b* is the shape parameter, similar *b* between formulations reflecting similarity in the underlying release mechanisms, and "*t*" is time.

Classical IVIVC and Internal Validation

The formulation fast (6 mm) was not included in the setting of IVIVC and kept for external validation.

A classical level A linear correlation was built linking *in vitro* dissolution Fd% and *in vivo* FDabs% input curves observed. A common linear regression for medium, and slow formulations was established linking FDabs% as a function of Fd% directly. For validation, the regression function was then applied to regress the *in vivo* FDabs%_pred on the corresponding three (mean) *in vitro* dissolutions. The convolution was applied to calculate the corresponding plasma concentrations and three sets of $C_{\max}$ and $AUC_{(0-t)}$ for fast, medium, and slow. Prediction errors percent (PE%) were then obtained for each formulation rate as follows:

$$\mathrm{PE}\% = 100\frac{|P_{\mathrm{obs}} - P_{\mathrm{pred}}|}{P_{\mathrm{obs}}} \tag{2}$$

where P are the observed (P_{obs}) and IVIVC model-predicted (P_{pred}) *in vivo* PK metric ($C_{\max}$ or AUC).

Internal predictability is acceptable when average percent prediction error is below 10% for $C_{\max}$ and AUC and none of the formulations have a prediction error greater than 15%. External predictability will be accepted if, with this new formulation, average percent prediction error is lower than 10% for $C_{\max}$ and AUC. In case of average percentage prediction error being between 10 and 20%, results will be considered as inconclusive and additional sets of data will be needed. If average percentage prediction error is greater than 20%, the predictability is inadequate and IVIVC must be revised (1,2).

Levy Plotting

As for the IVIVC, the Levy plot was established on the principle of all except one formulation used to establish IVIVC. The fast formulation (6 mm) was not included in the setting of IVIVC and kept for external validation.

Levy plotting was conducted to explore the time scale between *in vivo* and *in vitro*. The usual and empirical approach to build the Levy plot is to explore the observed Fd% and fraction (of bioavailable dose) absorbed (FDabs%) and to try to match them by proximity capture of the corresponding two times and create the plot axes. A relationship between *in vitro* and *in vivo* times corresponding to similar dissolution/absorption fractions is created and represented graphically in a time *in vitro vs*. time *in vivo* plot where regressions can be applied.

Inverse Release Function (IRF) Time Scaling for IVIVC and Internal Validation

Instead of using a regression of a classical Levy plot to link the *in vitro* to *in vivo* release times, an IRF method was applied for each formulation. The IRF provides the (equivalent) *in vitro* dissolution time as a function of FD%, the *in vivo* fraction released, absorbed, and reaching systemic circulation. It calculates time at which Fd% is equal to any given FDabs%. Figure 1 depicts a schematic of the method.

In the example, inverse Weibull was used as follows

$$\begin{aligned} t_{eq.InVitro} &= InvWb(\mathrm{Release}) \\ &= -Ln\left[\frac{-FD_{abs}\%}{F_{inf}} + 1\right]^{\frac{1}{b}} \cdot MDT \end{aligned} \tag{3}$$

where "Release" refers to *in vivo* drug release equivalent to FDabs%, as discussed above. Other parameters are as defined in the *in vitro* section.

In the scope of all except one approach the IRF was applied for medium (7.5 mm) and slow (9 mm) formulations. The average of the resulting $t_{eq.\ InVitro}$ from medium (7.5 mm) and slow (9 mm) was then used as the common time scale for the three formulations (fast (6 mm), medium (7.5 mm), and slow (9 mm)). A common regression was established by plotting time scaled Fd% *versus* FDabs% and used for fast, medium, and slow formulations.

The IRF time scaling, unlike classical linear first-order polynomial based "stretching" of the times *in vitro* to match those *in vivo* (rarely also vice versa), work the time scale implicitly via conversion of times into equivalent times *in vitro*. Therefore, final average scaled times may still differ in terms linear scale (*i.e.*, $t_{eq.\ InVitro}$ may still appear shorter than *in vivo*) and this is reflected, *e.g.*, in post IRF Levy plots.

For validation, the Weibull parameter estimates from *in vitro* profiles of three formulations were used to predict equivalent fraction absorbed *in vivo* FDeq% at IRF estimated times, $t_{eq.\ InVitro}$, by applying Eq. 3 as follows:

$$FD_{eq(t)} = F_{inf} \cdot \left[1 - \exp\left(-\frac{t_{eq.InVitro}}{MDT}\right)^{b}\right] \tag{4}$$

This predicted fraction absorbed *in vivo*, FDeq%, was used to obtain three corresponding *in vivo* PK profiles by convolution. The PE% for $C_{\max}$ and AUC were calculated as above: fast (6 mm) as external predictability and medium (7.5 mm) and slow (9 mm) as internal predictability.

RESULTS

The comparative bioavailability study *in vivo* plasma concentrations of drug with corresponding FDabs% *versus* time for three tests (fast 6 mm, medium 7.5 mm, and slow 9 mm Egalet® hydrocodone) formulations and the IR as well as their corresponding FDabs fraction (for two formulations used to establish the IVIVC) are shown in supplemental 1. The average *in vitro* dissolution profiles for the three test compounds are illustrated in supplemental 2.

The fraction absorbed was compared to fraction dissolved for test formulations using a classical IVIVC level A (Fig. 2). A linear regression, common for two tests formulations (medium and slow), was applied to predict FDabs% from Fd% based on this level A correlation for the two involved formulations as internal predictability (7.5 mm: medium and 9 mm: slow) but also to the remaining formulation (6 mm: fast) as external predictability.

In this first approach (*i.e.*, without application of the IRF time scaling method), FDabs% predicted from Fd% was used to obtain

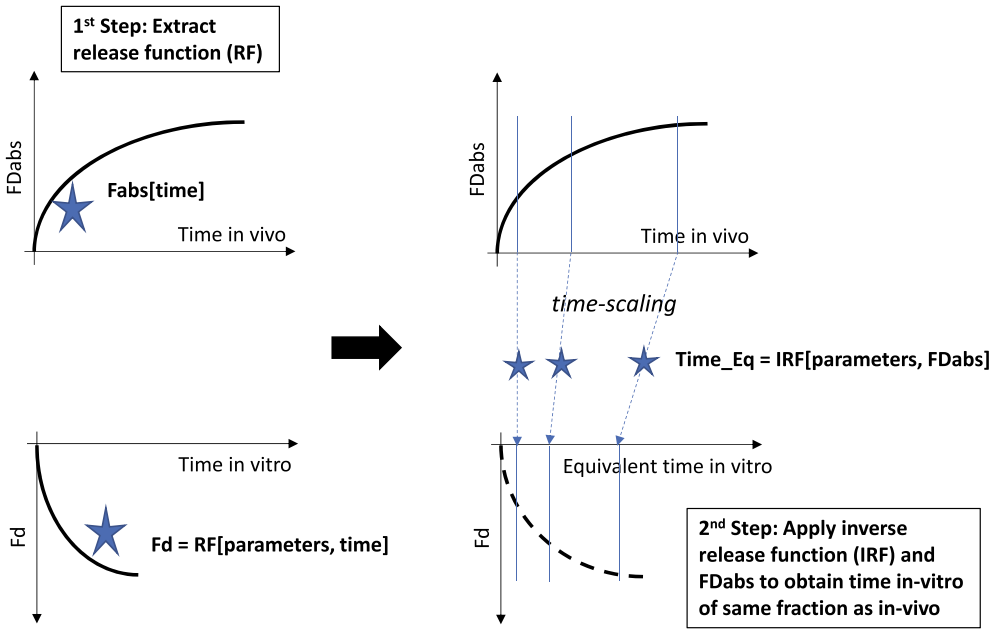

Fig. 1. Schematic of the process of transformation into equivalent *in vitro* time based on the inverse of the release function (IRF). First, the RF is obtained from the *in vitro* fraction dissolved (Fdis) profiles and so is the fraction absorbed *in vivo* (Fabs). Then, the latter is used in backwards estimation of the RF (IRF) to obtain the equivalent time points for an *in vitro* profile where the same Fdis = Fabs

three PK profiles by convolution and contrasted graphically (Fig. 3) *versus* observed PK of three formulations. The absolute prediction error (%PE) (Table I) for C_{max} and AUC between 1 and 25% resulting in average %PE of 20% for C_{max} (range −14 to −25%) and 4% for AUC (range −1 to −8%), average PE for C_{max} is greater than 10% and individual errors greater than 15%, so internal and external predictability was not acceptable according to regulatory guideline requirements. In addition, prediction results in a shift of Tmax values leading to inadequate shape of the curve. There was constant under-prediction at the absorption phase peak and under-prediction of the elimination phases for all formulations. An improvement of predictive error of estimates was pursued by applying IRF-based time scaling.

Levy Plot

The pre-IRF application empirical Levy plot as classically performed (manually or by specialized commercial software or custom written code as is the case here) is displayed in Fig. 4 for two internal validation formulations. The algorithm performs a search at each level of FDabs% minimizing the distance with the Fd% values and extracts the

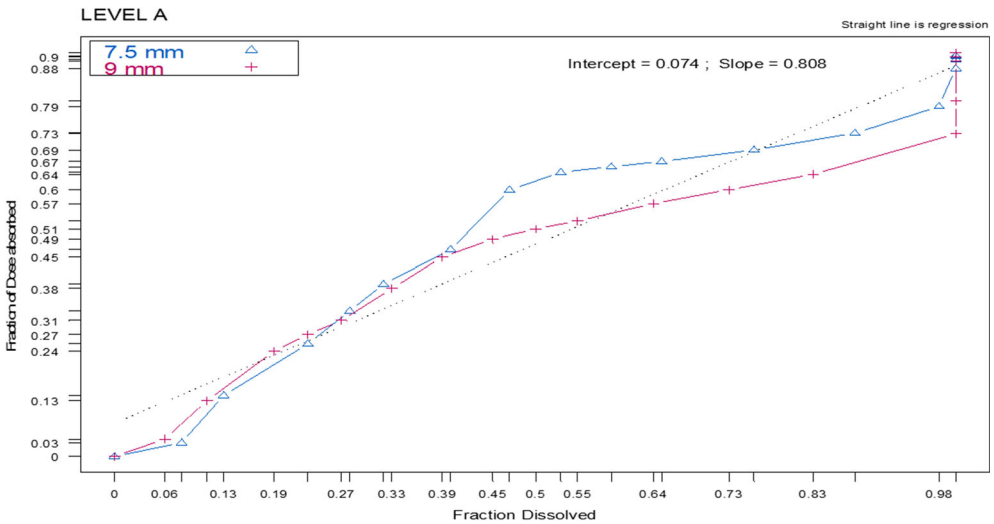

Fig. 2. Level A IVIVC without time scaling using two formulations (7.5 and 9 mm)

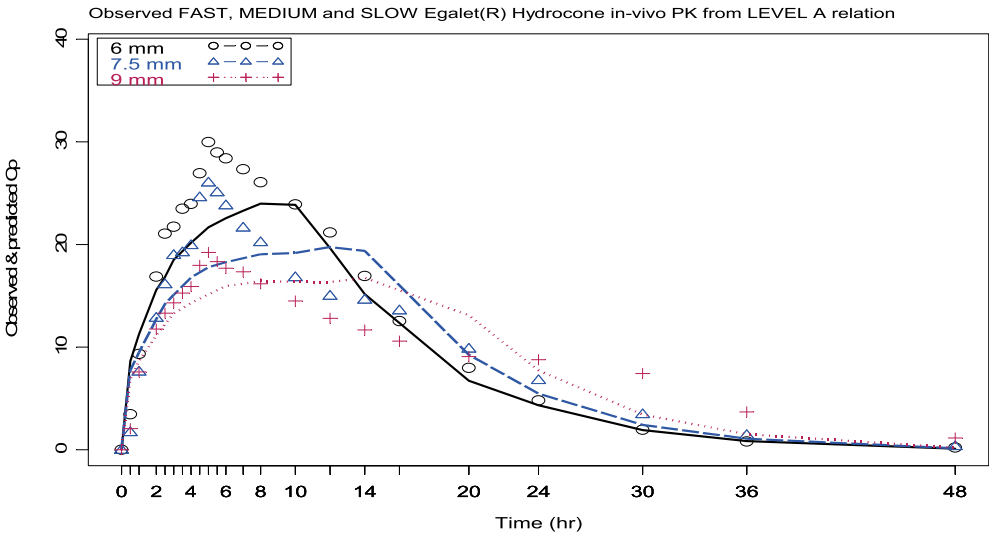

Fig. 3. First approach without time scaling prediction for the three test products of Egalet® by convolution based on an apparently linear level A prediction. Formulation of 6 mm (open circles, solid black line) external predictability, formulations 7.5 mm (open triangles and blue dashed line) and 9 mm (crosses and dotted red line) internal predictability

corresponding pair of *in vivo* and *in vitro* times, producing the Levy plot. The search interval can be adjusted manually or by the algorithm, *i.e.*, alike a two-dimensional simplex minimization.

Although there is a clear biphasic release situation, rates are similar, so common time scaling can be applied. Time scaling is needed as seen in the deviation from the unity line. However, it is also revealed from the reduced time point resolution that the approach is a rough approximation and that a significant amount of data points was excluded. Only 9 out of 21 time points were used. Non-linear erosion behavior of the formulation *in vivo* as a function of location (time in the gastrointestinal tract) results in non-optimal *in vitro* to *in vivo* PE% with the Levy plot approach (curves not displayed, average PE of 16% on C_{max}) even if better than the direct level A regression shown above.

IRF Time Scaling

Using the mean dissolution profile to characterize via modeling, the cumulative release process is straightforward with an adequate number of dissolution points, minimal analytical errors, and low variability resulting in good estimation of release model parameters. The Weibull was tested as the release function (Eq. 1) and was found to best represent dissolution profiles. The model parameters were obtained for three formulations (Table II). Percent dissolved was fixed to 100% at a maximum.

The formulation specific IRF, which in this case was an inverse Weibull function (Eq. 3), was then used to estimate the *in vitro* time that leads to a defined percentage (assigned based on each *in vivo* FDabs%) for medium and slow formulations (fast formulation was kept for external validation). This approach allows to directly estimate (without any minimization process in comparison to previous Levy's plot) *in vitro* time where percentage dissolved is the same as percentage absorbed. The *in vitro* time estimates per formulation are finally averaged to apply a common time scale as presented in Table III (mean time). In this case, *in vitro* values were represented along the *Y*-axis (as calculated), and *in vivo* values plotted along the *X*-axis (as observed) for the Levy plot. Figure 5 compares the reverse (*in vitro* – predicted

Table I. Prediction Errors for First Iteration (Standard Level A Regression) Internal Validation of Fast, Medium, and Slow Formulations *In Vivo* PK (corresponds to Fig. 3)

	Formulation type	Observed	Estimated	Prediction error (%)
C_{max} (ng/mL)	Fast (6 mm) External	30	24	−21
	Medium (7.5 mm) Internal	26	20	−25
	Slow (9 mm) Internal	19	17	−14
AUC [0–last] (ng h/mL)	Fast (6 mm) External	435	398	−8
	Medium (7.5 mm) Internal	404	398	−2
	Slow (9 mm) Internal	402	398	−1

C_{max} maximum concentration, *AUC* area under the curve

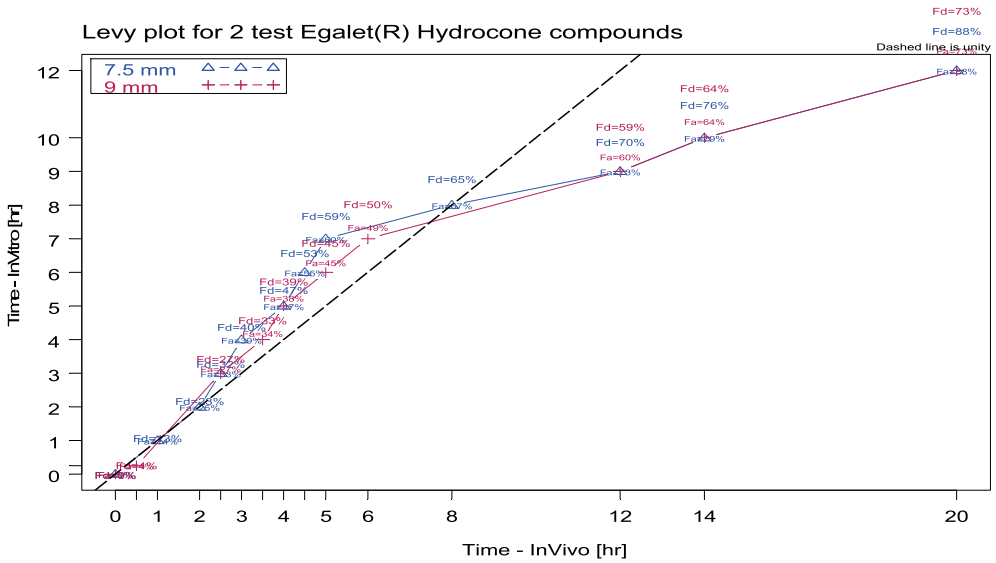

Fig. 4. Reverse Levy plot (*in vitro* time is predicted) for two of the test products prior to any scaling, straight line is unity

versus in vivo times) Levy plot both from the first approach comparison. There is improvement in coverage of the relative times but as discussed above the IRF differs from polynomial "stretching" of times in that time scales implicitly include the non-linearity in the actual cumulative dissolution and absorption profiles also accounting maximally for potential biphasic responses *in vivo* that may not have an equivalent (in initial formulation development) in *in vitro* profiles.

The convolution method was applied to estimate corresponding plasma concentrations for the three formulations (6 mm: fast (external validation), 7.5 mm: medium and 9 mm: slow (internal validation)) based on the IRF approach.

The predictions for all formulations are depicted in Fig. 6. Prediction errors for C_{max} and AUC were estimated again for all three formulations after this final approach for internal validation and listed for medium formulation in Table IV. Applying average time scale correction results in acceptable prediction errors (C_{max} 3.7% (range − 8 to + 1%) and AUC 4.2% (range − 5 to + 5%) as mean). The Tmax predicted were corresponding to Tmax observed indicating in addition that curve shapes are coherent with observed values. The improvement in the estimated profile fit *versus* observations and in prediction errors for C_{max} and AUC illustrates the benefit of applying the IRF time scale correction over alternative direct correlations. After IRF time scaling, the IVIVC becomes a strict 1:1 relationship: slope = 1 and intercept = 0.

Table II. Weibull Model Parameters for Three Mean *In Vitro* Formulation Profiles

Formulation	Parameter	Estimate
Fast (6 mm)	MDT	5.53
	b	1.11
Medium (7.5 mm)	MDT	10.18
	b	0.98
Slow (9 mm)	MDT	31.87
	b	0.84

MDT mean dissolution (or release) time

DISCUSSION AND CONCLUSION

In an ideal situation of IVIVC, the *in vitro* dissolution method is able to mimic *in vivo* dissolution conditions, so the fraction absorbed per time is interchangeable with fraction dissolved in time leading to a so-called 1:1 level A IVIVC. Trying to adjust the dissolution to mimic *in vivo* times is not always possible and can be attempted only when the difference between *in vitro* and *in vivo* for time scaling is not large. The approach of adapting a dissolution method to

Table III. Inverse Release Function (IRF) Calculated Absorption Equivalent Times for *In Vitro* Release

Time *in vivo*	Medium 7.5 mm	Slow 9 mm	Mean time scaling
0.0	0.0	0.0	0.0
0.5	0.2	0.3	0.3
1.0	1.1	1.2	1.2
2.0	2.3	2.6	2.5
2.5	3.1	3.2	3.1
3.0	3.8	3.6	3.7
3.5	4.3	4.1	4.2
4.0	4.8	4.8	4.8
4.5	6.0	5.5	5.7
5.0	6.8	6.0	6.4
5.5	7.3	6.5	6.9
6.0	7.5	6.8	7.2
7.0	7.8	7.2	7.5
8.0	8.0	7.6	7.8
10.0	8.5	8.4	8.4
12.0	9.3	9.0	9.2
14.0	10.6	9.8	10.2
16.0	11.8	10.5	11.1
20.0	12.9	12.0	12.4
24.0	13.6	13.9	13.7
30.0	13.8	16.4	15.1
36.0	13.8	16.7	15.3
48.0	13.9	16.9	15.4

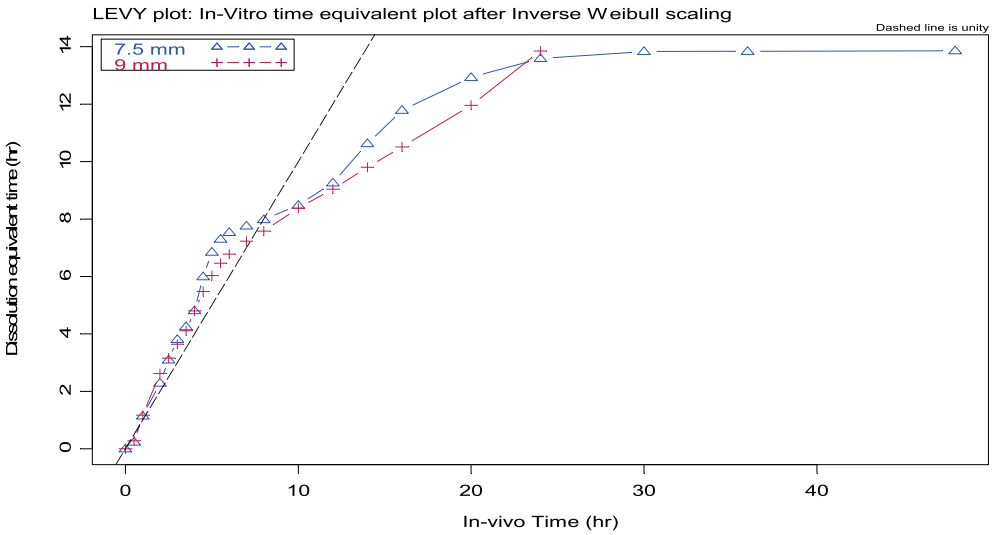

Fig. 5. Reverse Levy plot (*in vitro* time is predicted) for two of the test products after IRF scaling

fit *in vivo* times would not be feasible, for instance, for implants. The release *in vivo* is months or even years and the dissolution must be shortened to days. In these situations, and for many oral extended-release products, the only realistic approach within the context of level A correlations is to apply a time scale correction.

The Levy plot investigates the relation between two times linked to the same release result, *e.g.*, dissolution and absorption fraction or percent. Overall, this approach is used to find the relationship between different *in vitro* and *in vivo* time scales rather than as a base for an IVIVC. It allows to adjust and find the best time scaling between the data and to determine if both sets of data exhibit similar release mechanisms.

However, loss of information is a usual challenge in such direct time scaling due to limited sampling points and the difficulty of matching similar levels of absorbed and dissolved fractions. The potential for improvement of the IVIVC by applying time scaling is hampered by this elimination of information that leads to reduction in quality and robustness of the IVIVC. Alternatively, missing points are commonly estimated using linear interpolation leading also to approximation particularly when *in vitro* sampling is not dense enough.

Pharmacokinetic *in vivo* sampling points to characterize plasma concentration profiles; hence, the derived cumulative fraction absorbed *in vivo versus* time are usually limited by ethical or protocol reasons. Dissolution sampling times could be limited for practical reasons but the data are less subject to noise than the *in vivo* data which is subject to error linked with bioanalytical methods, intra-subject differences, and inter subject variability. This further complicates the direct

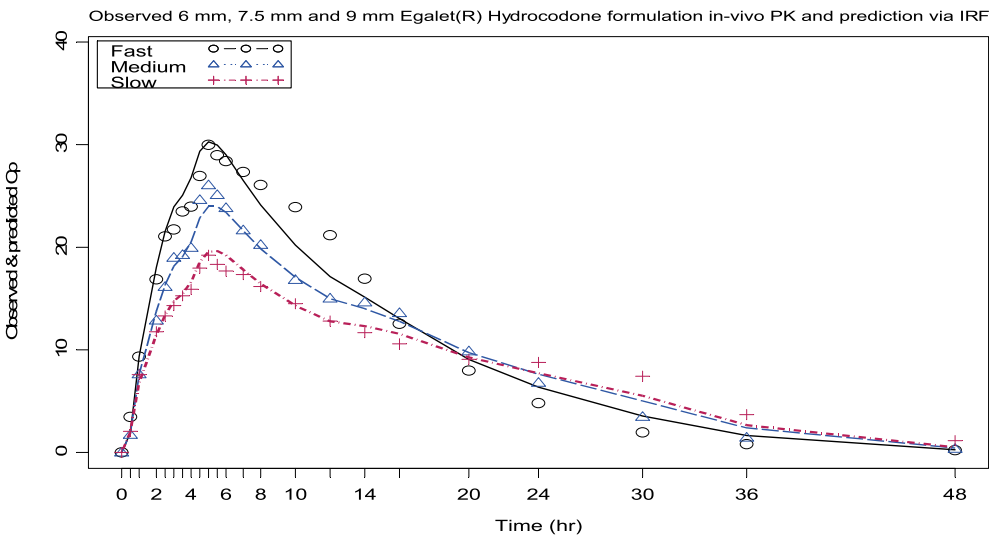

Fig. 6. Overlay of observed and predicted *in vivo* drug concentration *versus* time PK after convolution of IRF estimated equivalent *in vitro* times and then fractions absorbed *in vivo*. Formulations 7.5 mm (triangles and dashed line) and 9 mm (crosses and dashed-dotted line) are for internal predictability and formulation 6 mm (open circles solid line) is external predictability

Table IV. Prediction Errors for Final Iteration Internal Validation of Medium and Slow "Speed" Formulation *In Vivo* PK (corresponds to Fig. 6.) After Application if IRF Time Scaling

	Formulation type	Observed	Estimated	Prediction error (%)
C_{max} (ng/mL)	Fast (6 mm) External	30	30	1
	Medium (7.5 mm) Internal	26	24	−8
	Slow (9 mm) Internal	19	20	2
AUC [0–tau] (ng h/mL)	Fast (6 mm) External	435	449	3
	Medium (7.5 mm) Internal	404	424	5
	Slow (9 mm) Internal	402	384	−5

C_{max} maximum concentration, AUC area under the curve

comparison between *in vitro* and *in vivo* just as discarding, skipping, or averaging time points in the classical time-to-time matching in IVIVC leads to loss of valuable information.

The best and safest approach to link *in vitro* with *in vivo* results is to program a large number of dissolution samples for example with an on-line automated sampling and analysis system. In such a scenario, interpolation between two dissolution points could be as simple as linear interpolation and would yield similar results to more sophisticated modeling methods. In the other extreme, when only data from very limited sampling time points is available, the equations to model dissolution behavior may be overparametrized leading to potentially inaccurate predictions. The optimum situation is when sufficient sampling points are included to reliably model dissolution behavior. Lately, mixed effects methodologies are increasingly applied to estimate dissolution fraction model parameters potentially circumventing the sparse time point complications.

Fitting *in vitro* cumulative dissolution fractions with a model such as Weibull opens the possibility of increasing the precision and accuracy of level A IVIVCs when correction for different time scales is required. Modeling of dissolution data is not uncommon as it is used to characterize formulation dissolution behavior and is also used to compare dissolution behavior in different conditions such as the impact of different temperatures in accelerated conditions (12).

Weibull functions are commonly implemented in numerous commercial IVIVC software for modeling dissolution profiles (Phoenix, Gastro+, etc.) offering flexibility when fitting data across a range of dissolution "shape", exponential from $b \leq 1$ up to sigmoid curves at $b >> 1$. Other models such as first-order dissolution profile fitting in order to estimate fractions dissolved at any *in vitro* time have been recently mentioned in the context of IVIVC establishment (13,14).

An important advantage of modeling the dissolution profile is that all of the available *in vivo* data can be linked with dissolution with higher precision than when dissolution data is not modeled. The inverse release function (IRF), defined as the inverse of the dissolution release function (*e.g.*, Weibull in our example), is a direct and efficient way to establish the time link capturing all relevant information precisely.

Once the correlation is established with the test formulations, it can be used to estimate a new target test dissolution profile from a known formulation of interest (*e.g.*, a reference formulation to be mimicked). The target cumulative fraction absorbed (FA%) *versus* time profile can be established by deconvolution of the known reference product plasma concentration profile or the ideal product profile designed based on PK/pharmacodynamic considerations. Then, the release function (*e.g.*, Weibull) of the most promising test product rate can be used to estimate what the optimal target dissolution profile should look like based on that FA% and the IRF method.

Time scaling may be used as long as the time scaling factor is the same for all formulations. If the Levy plot shows different *in vivo-in vitro* time relationships per formulation, then this is probably related to an absence of a common release behavior. Different time scales for each formulation indicate absence of an IVIVC. This is the usual situation when the formulations do not share the same release mechanism. For example, one releases the drug substance predominantly by diffusion whereas erosion mechanisms explain the release of another formulation. In such a situation, the Levy plot provides an indication that the dissolution method is reflecting the *in vivo* behavior of one of the two formulations only. If the formulations share the same release mechanism, which can only be ascertained by the team that developed and manufactured them by the same process, with the same excipients and drug substance, the dissolution method does not represent the *in vivo* behavior and the *in vitro* tool must be reexamined.

Non-linearity in a Levy plot implies a difference in the kinetic order of the conditions compared, which in the IVIVC context are *in vivo* and *in vitro*. For example, *in vitro* dissolution may appear to follow zero order kinetics and *in vivo* input first order. Optimization of the dissolution method to reflect the same order of release kinetics as *in vivo* data would be the best option in order to represent similar release mechanisms *in vitro* and *in vivo*. Another complication may arise when the observed release rate is not constant, *e.g.*, as the formulation progresses through the gastrointestinal tract. The best way to proceed is by investigating why the

release kinetics observed *in vivo* could not be reproduced *in vitro*. If a biphasic relationship exists then the time of phase change is of importance and must be evaluated according to physiology in conjunction with the type of formulation. For an erodible matrix such as Egalet®, used here, a time of phase transition at approximately 3.5 to 4.5 h after oral administration in fasting conditions could translate as the time at which the tablet passes from the small to the large intestine, as previously addressed (10) and confirmed by pharmacoscintigraphic evaluation (11). In the same line of thought, a matrix tablet with a pH-dependent release rate will show a biphasic Levy plot related to the point of *in vivo* pH changes. Approaches that take into account dissolution rates at two pH's (15) may be considered but this goes beyond a classical level A IVIVC. If a plausible explanation for the non-linearity cannot be identified but is the same for all formulations, *in vivo* can still be predicted based on *in vitro* assuming a black box model. Black box approaches carry the risk that seemingly irrelevant changes in formulations translate into unexpected *in vivo* profiles as the critical factor behind the *in vivo* behavior is unknown.

The level A approach, discussed here, applying an IRF concept for time scaling can be used for any drug and delivery system as long as a single dissolution method represents the *in vivo* release and there is a common release mechanism across formulations. This includes any oral modified release product with pH-independent release mechanisms. In case of pH-dependent release, IVIVC establishment becomes complex due to the influence of gastric emptying times on the pharmacokinetic profiles and differential equation-based methods may have to be pursued for IVIVC establishment. The IRF approach can also be pursued in case of limitations introduced due to absorption windows as long as the total amount absorbed is similar for all the release rates under evaluation (*i.e.*, similar relative bioavailability between formulations). Level A IVIVC establishment of extravascular delivery systems such as intramuscular depots, formulations for vaginal delivery, transdermal systems, and others would also be refined with the IRF approach. The only limitation is of course the need of common release and absorption mechanisms among formulations as well as a single dissolution method that represents *in vivo* release rates with the application of time scaling.

Here, use of the inverse Weibull function for interpolation of dissolution time data and comparison with a classical level A correlation approach has been illustrated through a simple real example. The method is generalizable to any release function, other than Weibull, so that it is essentially an IRF method. One limitation may be the preference for an analytical inverse of the function used to describe the dissolution. Avoiding over parametrization of the dissolution equation could also be a challenge in that context. However, in cases of non-analytical IRF, numerical methods could be applied to provide a solution.

It is important to note that successful time scaling should result in a 1:1 IVIVC if a single absorption process is involved. After time scaling, the linear regression of the Fd% obtained from time scaled times *vs*. FD% returns a slope close to 1 and an intercept close to 0, at least across different release rate sections. Time scaling could then be considered as a full surrogate of the classical IVIVC approach. Any further adjustment using, after the time scaling, a classical IVIVC would only improve marginally the results but would complicate greatly the process.

An advantage of the IRF method is that all the *in vivo* time points (and their associated parameters) are directly related to the corresponding time *in vitro* (see Table III first and last columns). Any *in vitro* dissolution profile, within the studied formulation and method, could then be directly translated via convolution into a PK input function. This method could also help to optimize the *in vivo* sampling points. The critical aspect of *in vivo* bioavailability or bioequivalence studies is to sample accurately in order to capture (i) the C_{max} (it must not be the first sampling point) and (ii) to characterize adequately the curve where the maximum variation is expected *in vivo* such as the absorption phase. Using this approach, knowing the *in vitro* performance of a formulation, the cumulative percent dissolved and the associated cumulative *in vivo* release fraction at each time point, the *in vivo* impact of *in vitro* variation can immediately be anticipated and predicted. Additionally, with a non-linear Levy plot from a biphasic relationship indicating, *e.g.*, an inconsistent behavior of the formulation between *in vitro* and *in vivo*, the IRF method could be applied to optimize the dissolution test. Knowing the *in vitro* time at which the difference first occurs and the correction factor given by the slope of the Levy curve, the rotation speed, for example, could be adjusted in such a way that the Levy plot becomes linear leading to a totally biopredictive dissolution reflecting exactly the same phenomenon as *in vivo*. This information can also provide insight for the development of other dissolution methods using alternative apparatus to classical USP I or II (such as USP III or USP IV) by indicating when a media or rate (dip per minute or flow rate) change would be needed to address non-homogenous release through the gastrointestinal tract.

Here, a typical formulation development situation was presented with three increasing release "rate" test compounds tested in a comparative bioavailability trial *versus* a reference product for a sustained release product. The IVIVC with and without time scaling was applied using the "all except one" concept as follows: Two formulations were used to establish IVIVC and for internal validation and the third formulation was used as external validation. The formulation used for external validation followed the same release mechanisms but was outside of the *in vitro* and *in vivo* limits used to establish the IVIVC providing an extreme test of robustness. This confirms the IVIVCs ability to predict *in vivo* PK profiles from *in vitro* release profiles without iteration and provides the extreme dissolution profile for which the ability is validated. In order to insure the robustness of the IVIVC, it is possible to select randomly the formulations used for internal and external predictability.

In conclusion, the IRF method was applied to obtain the *in vitro* equivalent times of dissolution and the convolution prediction was repeated this time with near negligible prediction errors. Exploring the time scale, via Levy plots or the IRF, allows to define a common time scale correction that can improve the correlation between fraction absorbed and fraction dissolved. Modeling *in vitro* dissolution data and the use of the inverse of the modeling function (IRF method) is a straight forward approach, using no minimization processes, to establish IVIVC when correction for time scaling is required.

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