REVIEW

IN VITRO IN VIVO CORRELATION BASIS AND APPLICATION TO SLOW RELEASE INJECTABLE FORMULATION, A REVIEW

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Abstract

The aim of the present paper is to present the basic knowledge about *in vitro in vivo* correlations (IVIVC). The various definitions are given as well as the requirements to establish IVIVC. The basic concepts and IVIVC use and limitations are described. Application to slow release injectable formulations is presented at the end of the paper as a particular example of IVIVC.

Rezumat

Scopul lucrării este de a furniza informaţiile de bază legate de corelaţiile *in vitro-in vivo* (IVIVC). Sunt prezentate cerinţele specifice pentru stabilirea IVIVC, conceptele de bază, utilizările şi limitările acestora. Aplicaţii în cazul formulărilor injectabile cu cedare lentă sunt de descrise ca un exemplu particular de IVIVC.

Keywords: biopharmacy, IVIVC, slow release, injectable formulation

Introduction

The term *in vitro in vivo* correlations (IVIVC) was introduced in the late 1950th by Wagner *et al* [65]. In the last 50 years many papers have been published in this domain, particularly in the late 80th early 90th, showing a growing interest from academia and pharmaceutical industry [2, 3, 7, 32, 53]. This interest was also supported by regulatory authorities; several countries published guidelines describing IVIVC and recommendations for its development [20-22, 29, 56-59, 61]. Definitions of *in vitro in vivo* correlations were proposed by the International Pharmaceutical Federation (IFP), the United States Pharmacopeia (USP), the US Food and Drug Administration (FDA), the European Medicinal Agency (EMA), International Conference for Harmonization (ICH) and World Health Organization (WHO). All those definitions are globally similar and can be summarized as relationships observed between parameters or curves derived from *in vitro* dissolution and *in vivo* absorption or bioavailability / bioequivalence parameters.

Three different levels of correlation are described in USP: A, B and C. Level A, or point to point correlation, aims to establish a link between the full *in vitro* dissolution curves and the full *in vivo* absorption curves. Level B, uses parameters derived from *in vitro* curves and *in vivo* profiles, such as statistical moments (e.g., *in vitro* corresponds to mean dissolution time and *in vivo* refers to mean residence or absorption time). Level C, or single point relationships, uses dissolution parameters such as % of drug dissolved at a given time and pharmacokinetic parameters mainly linked to drug exposure like maximum concentration (C_{max}) or area under curve (AUC) [7-12, 19, 56]. The underlined assumption with these correlations is that the limiting factor *in vivo* must be reproducible *in vitro* and should not be linked with physiological factors (i.e., permeability/permeation through the membrane), instead, with different rates of drug release from the drug dosage form (DDF) or solubilisation of the active pharmaceutical ingredient (API). All these correlations imply linear non-saturable pharmacokinetic processes [25, 35]. Figure 1 show step by step the LADME (liberation, absorption, distribution, metabolism and excretion) processes upon administration of a drug by oral route and all potential related processes [8].

For IVIV correlations, the absorption curves can be derived by various methods such as model dependent (Wagner-Nelson or Loo-Riegelman) or model independent numerical deconvolution [8, 25, 64]. After the appropriate calculations are performed, the input curve known as "absorption" curve is presented through the percent of the fraction of dose absorbed *vs.* time (% FD: from 0 to 100%, F being absolute bioavailability). This absorption curve represents the slowest of all the phenomena that take place prior and during absorption, such as drug release

from the pharmaceutical form, dissolution in the gastro-intestinal fluid, and permeability through intestinal membranes (Figure 1). To obtain a good estimation of the absorption curves, a sufficient number of well positioned samples are required. If the absorption is slower than the elimination, a flip flop phenomenon could be anticipated and attention must be paid to the PK parameter calculation in order to prevent any data misinterpretation. In the case of flip flop kinetics, the parameters of interest for bioequivalence (BE) studies and IVIV correlations are modified: AUC from 0 to infinity and absorption rate are overestimated. To avoid such a problem, it is recommended to compare the apparent terminal half-life between concentration–time curves obtained using an immediate release (IR) or an intravenous (IV) and slow release (SR) formulation [12] and to use the IR or IV one.

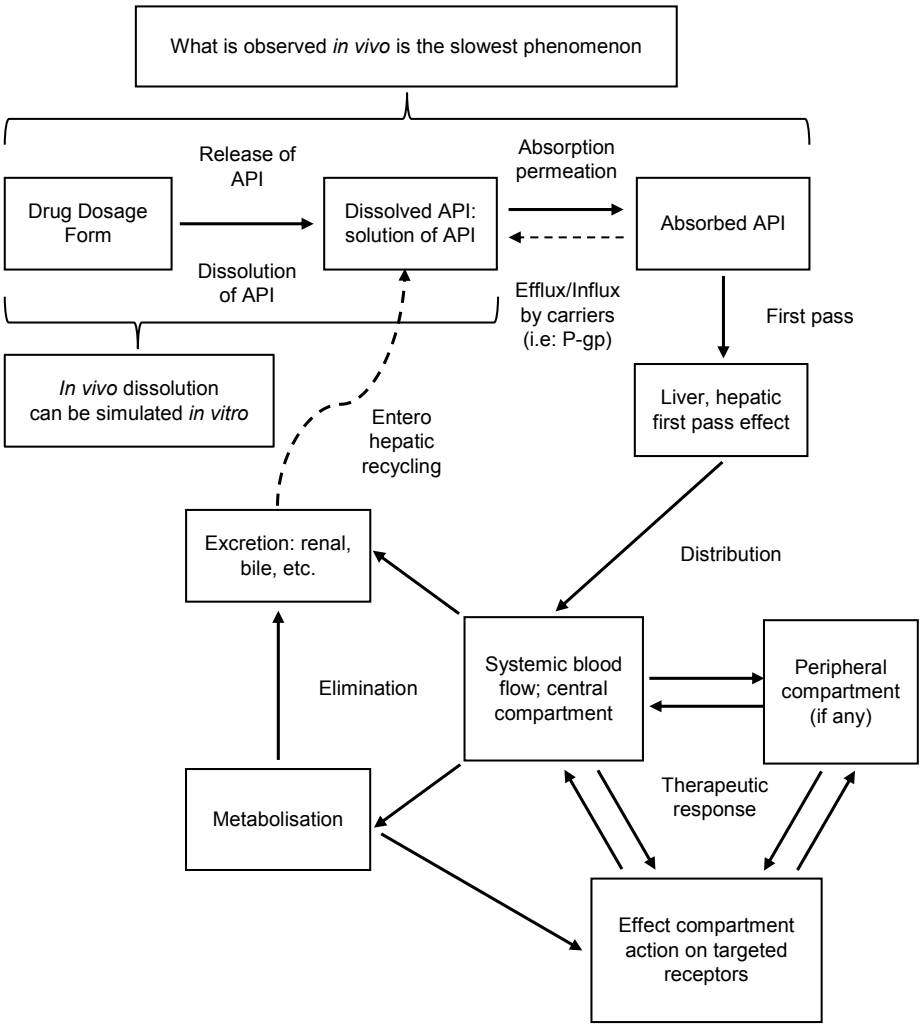

Figure 1.
Behaviours of the drug after *per os* administration from [8]

IVIVC stressed the importance of the *in vitro* dissolution [2-3, 9, 46, 55] which reflects directly the release and dissolution of the drug from the dosage form and all the aspects related to the formulation process as presented in Figure 2. In contrast to *in vivo* studies, *in vitro* methods are less "standardized" as mainly USP apparatuses 1 to 4 could be used with various media (hydrochloric acid, simple buffer, surfactant or enzymes could be added, etc.) under pre-established technical parameters (i.e. volume, rate, flow). Any method (combination of apparatus, dissolution media and flow rate or rotation speed) to discriminate between formulations can be used. In order to compare the results, the same method must be used for all the formulations to compare. The analysis of the dissolution curves (percent API dissolved vs time) will allow the calculation of parameters like time to 10, 50 or 90% of API dissolved [T10, T50, T90%), Dissolution Efficiency (DE), Mean dissolution time (MDT) [3, 18, 46], etc.

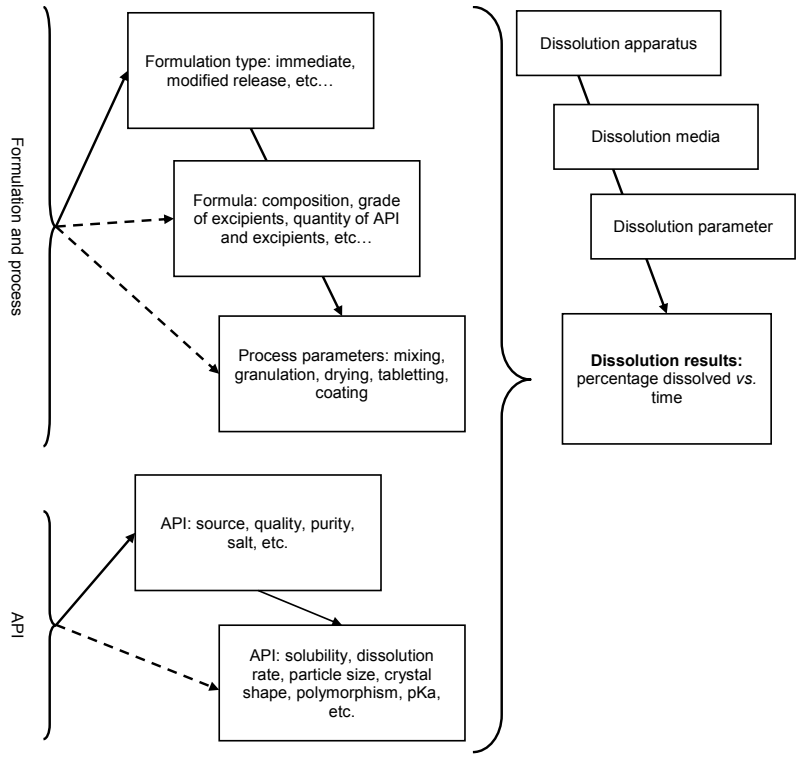

Figure 2.
Dissolution as a reflection of the pharmaceutical complexity of the dosage form, solid line: directly accessible information, dashed lined: underlined information (from ref 9). MR: modified release; IR: immediate release; API: active pharmaceutical ingredient.

IVIVC are performed when either the drug release from the pharmaceutical form or the solubilisation of the drug are the limiting factors for absorption. In this case, formulation process plays a key role in the development of the dosage form and IVIVC are of great interest. Drug release is the limiting factor when solubility (or dissolution rate of the API) and permeability are faster than drug release. If the drug dissolution rate is the limiting factor, IVIVC could be established, however, its interest is limited as minor changes in physical chemicals properties (e.g., like particle size, etc.) could affect the *in vivo* behaviour.
In order to proceed with IVIVC, all the pharmacokinetic phenomena must be linear and non-saturable in the range of application of the formulation. In case of nonlinear pharmacokinetics (saturable metabolism or absorption, active transport mechanisms) or high variability of the pharmacokinetic profiles (HVD), the interest of IVIVC is limited as the modifications of the formulation might not be observed *in vivo* due to the magnitude of the physiological processes mentioned above that would supersede any formulation effort. Similarly, all the IVIVC work performed is only valid in the context of the studied formulations, in other words, all the modified and optimized dosage forms must be manufactured within the same company, with similar processes and have the same release mechanism and route of administration [10, 11]. For instance, it would not be acceptable to use the IVIVC generated on coated microspheres to extrapolate and optimize a hydrophilic matrix tablets or to use the IVIVC generated on the originator formulation to submit a generic dossier.

Establishing IVIVC

When either drug dissolution or release from the pharmaceutical form are the limiting factors and no other absorption limiting processes take place, IVIVC could be investigated. Level A IVIV correlation is considered to be the most informative and the recommended one, when possible, by health authorities.
As stated by the FDA and EMA [20-22, 56-59] two approaches can be used to establish a level A correlation. In the classical approach the process is decomposed in 2 main steps: calculation of the *in vivo* input function (absorption) by a deconvolution technique and then link with *in vitro* dissolution data using a regression method [6, 11, 16]. An alternative method would be to use direct convolution techniques. Bayesian or neural network approaches are proposed [5, 15, 16, 24, 25, 30, 39, 47, 55] allowing the investigator to treat all the information in a single process using specific algorithms and software.

After the IVIVC is established, its ability to predict accurately the *in vivo* data is verified, in order to assess the predictability. Evaluation of the predictability can be done by back calculation of the initial data utilized for the establishment of the IVIVC (internal predictability) or using a new data set (external predictability). Predictability is very important mainly when IVIVC is used as a surrogate of *in vivo* data (biowaiver). Figure 3 describes the two types of predictability, internal and external.

Internal predictability is based on the initial data used to develop the IVIVC model. In this approach, the IVIVC is established from a set of initial *in vitro* and *in vivo* data corresponding to one or more formulations (optimally at least 3 formulations with different release rate). Based on the IVIVC and the *in vitro* data, the initial plasma concentrations are back calculated and compared to the observed one. The internal predictability is considered to be conclusive [20-22, 56-59] if the average absolute percent prediction error (% PE, as the ratio of (observed-calculated) over the observed) is less than 10% for the main bioavailability parameters (AUC and C_{max}). In addition, the % PE for each formulation should not exceed 15%. If these limits for % PE are not met, an external predictability must be established and, if conclusive, will allow the use of IVIVC as *in vitro* surrogate of *in vivo* studies.

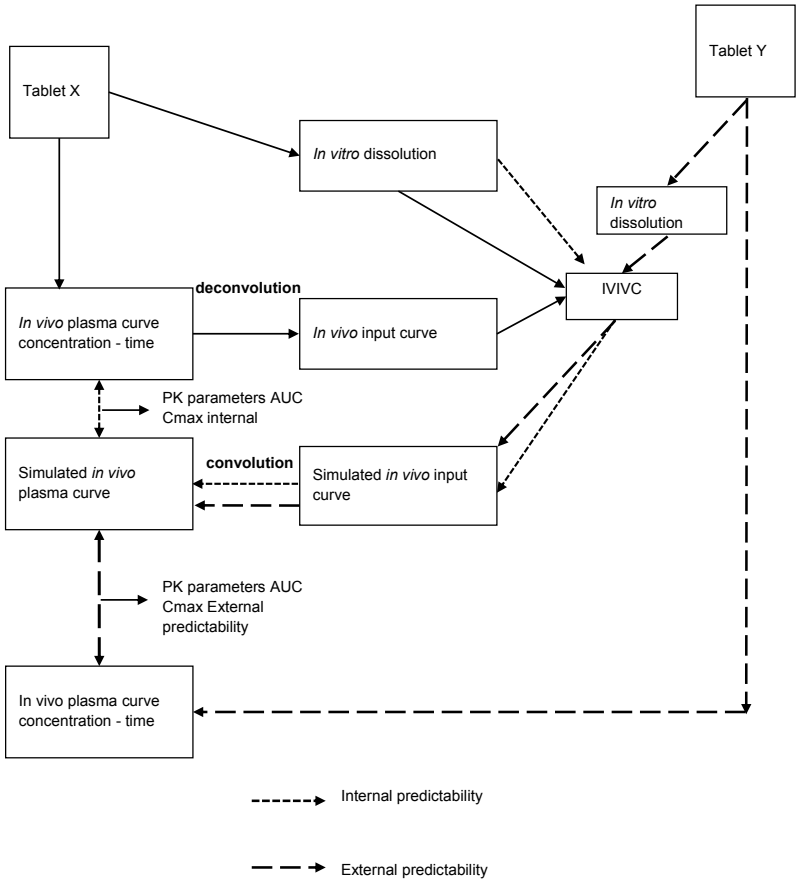

Figure 3.
Internal and external predictability of IVIVC

External predictability is based on the calculation/ simulation of plasma concentration curves of new formulations based on the defined IVIVC model. The IVIVC is established from a set of initial data *in vitro* and *in vivo*, corresponding to one or more formulations. Based on the IVIVC and the *in vitro* data of new formulations, the plasma concentrations over time are calculated and compared to the observed ones. The external predictability is considered to be conclusive [20-22, 56-59] if % PE is less than 10% for the main bioavailability parameters (AUC and C_{max}); a prediction error between 10 to 20% is considered inconclusive and further work is needed. Consistency in predictability is an important factor, thus % PE greater than 20% indicates usually an inadequate IVIVC. External predictability is often considered more powerful as a new set of data is used to check the effectiveness of the correlation.

Except for narrow therapeutic index drugs, a conclusive internal predictability is sufficient, external predictability is mandatory for narrow therapeutic index drug.

Using IVIVC

IVIVC and mainly level A IVIVC is a powerful tool in drug development that can be used in many different instances [10-11, 19, 20-22, 29, 32, 43, 56-59, 61]. It shows well understanding of the release characteristics of the pharmaceutical dosage form over time. IVIVC could be used as a process and quality control tool for the validation of the manufacturing specifications limits (dissolution limits). It facilitates the determination of specific regulatory demands in case of minor changes in formulation and can be used as a surrogate of certain bioequivalence studies for extended release formulations (biowaiver). Good examples of the latter could be a modification in the quantitative composition of the excipients, changes in the dose strength, and minor modifications of the manufacturing process, production site and scale up.

The ICH guideline for registration of pharmaceuticals for human use [32] and European and FDA Guideline on IVIVC [20-22, 56-59], emphasize the interest of IVIVC and its potential use for formulation optimization, dissolution limits setting, replacement of bioequivalence studies during product development, thus facilitating certain regulatory decisions. The IVIVC established for one formulation cannot be extrapolated to new dosages forms developed by a different sponsor or with different release mechanism. New manufacturing conditions or changes in pharmaceutical formulation will require development of a new IVIVC.

The development of IVIVC is a tool that allows to speed up drug development as it can be used to understand better the *in vivo* release mechanism, establish key factors of the formulation (Critical Quality Attribute) to optimize it through a Design of Experiment, and reduce the risks in bio-equivalence studies [5, 14, 23, 31, 33, 36, 37, 40, 42]. IVIVC may reduce the number of *in vivo* studies during drug development and can be established using forecasted studies (with no additional cost).

In absence of IVIVC, FDA and EMA guidance [20-22, 56-59] defined the dissolution limits for lots. For IR formulation often a single point is required, for ER or SR formulations at least 3 time points to determine fraction of drug dissolved over time are required to cover the early, middle, and late dissolution parts of the curve. Usually, the first time-point should be at approximately 20% of total drug dissolved in order to cover possible burst effect, the second around 50%, and the last one around 80-90% of drug dissolved. At any dissolution time point, the specification allows ± 10% deviation from the mean dissolution profile obtained from the clinical/bioavailability lots. FDA and EMA consider certain exceptions allowing a wider deviation range, which must be proven to be bioequivalent and reproducible.

In presence of IVIVC (level A) for ER or SR formulations, a minimum of three time points is recommended to establish the specifications; limits are based on the simulated plasma-concentration curves from the dissolution profile and on the established IVIVC. The upper and lower limits for the dissolution specifications should define bioequivalence and must result in a maximal difference of 20% between lower and higher predicted C_{max} and AUC [20-22, 56-59]. Those limits can serve as surrogate marker for *in vivo* behaviour and thereby confirm consistent performance of batches. Limits greater than 20% must be justified. IVIVC is a powerful tool to set up dissolution limits, those limits being particularly important in case of biowaivers [9, 18, 27, 28, 38, 41, 43, 53].

In case of level C IVIVC, this correlation can be used to help establishing dissolution limits. A single point Level C correlation allows dissolution specification to be set at one time point. In case of multiple points Level C, the limits should be covered for at least 3 points of dissolution (defined above). The limits are those described earlier in absence of IVIVC or, in case of prediction of PK parameters, not more than 20% difference in the predicted C_{max} and AUC.

IVIVC could also be used as a support of biowaiver. A biowaiver is the use of an *in vitro* dissolution curve in place of an *in vivo* study (as surrogate marker) to prove equivalence of dosage forms, manufacturing process or site. The use of biowaivers is based either on the physical-chemical properties and permeability of the studied drug according to the Biopharmaceutical Classification System, BCS (2), or whether an IVIVC has been established and validated, the latest being developed in the FDA SUPAC guideline [7-9, 19, 32, 37, 43, 61]. In both cases *in vitro* data are used as surrogate of *in vivo* studies.

According to FDA biowaivers are classified in 5 different categories:

- Category 1: Biowaivers without IVIVC
- Category 2: Biowaivers using IVIVC: non narrow therapeutic index drugs
- Category 3: Biowaivers using IVIVC: narrow therapeutic index drugs
- Category 4: Biowaivers when *in vitro* dissolution is independent of test conditions
- Category 5: Situations for which IVIVC is not recommended

EMA [20-22] and FDA [56-59] define in a similar way use of IVIVC as surrogate of *in vivo* studies. A biowaiver demands validated (predictability has been established) Level A correlation (mainly used for extended formulation), and could be applied in

case of major changes of non-release-controlling excipients, insignificant changes of release-controlling excipients, major changes in method or site of manufacturing. Several scenarios are considered under category 2 (Biowaivers Using an IVIVC: Non-Narrow Therapeutic Index Drugs) and category 3 (Biowaivers Using an IVIVC: Narrow Therapeutic Index Drugs). These scenarios within each category will define the situations in which IVIVC could be used as surrogate of *in vivo* data.
Category 5 does not recommend the use of IVIVC: difference of release mechanism between formulations, strength outside of the effective and safety limits, approval of new ER product based on the established IVIVC by a different sponsor or with a different API (i.e., generic application with IVIVC established with the reference product), and a formulation change implying excipients which affect gastro intestinal tract motility, absorption of studied drug or solubility.
Level C correlation cannot be used for biowaiver purposes unless a relation was found between all bioequivalence criteria and dissolution of all time points corresponding to at least early, middle and late dissolution (Multiple Level C) on all the bio-availability parameters (AUC and C_{max}). If a relationship is found between all PK parameters and dissolution parameters, a Level A correlation should be also investigated.

IVIVC for modified release injectable dosage forms

To prolong the action of drugs formulated as injectable dosage forms [7], two options are possible. First, modifications of the release rate from the formulation administered subcutaneously (S.C.) or intramuscularly (I.M.), this class contain implants and «depot» formulations. Second the modification of the distribution (and elimination) associated or not to modification of the release of the drug, often administered as intra venous (I.V.). The formulations combining modification of release, distribution and elimination are called vectors, like liposome or noisome. The modification of the elimination of the drug is obtained by modification of its characteristics (pegylation of the peptide for example). In case of modification of distribution and elimination, IVIVC cannot be applied as the release mechanisms are not the limiting factors. The only case in which IVIVC can be applied for injectable drugs is when the release is the only limiting factor used to sustain the *in vivo* plasma profile of the drug [7, 51]. The main representatives of this class are dosage forms which can release incorporated drug over weeks, months or years. In order to obtain *in vitro* dissolution data, very often accelerated release method is developed, with the aim to shorten time span to few days. As a difference exists in this case between the *in vivo* absorption and the *in vitro* release in term of duration, a time scaling must be applied. This operation can be handled using Levy's plot. Levy's plot is established by plotting *in vitro versus in vivo* times at user-specified dissolution/input (absorption) values. The *in vitro* values are set as X, as they are assumed to be less variable, and the *in vivo* values as Y. The equation is of the form Y=bX+a. The Figure 4 presents the typical plot with similar order but different rate between *in vivo* and *in vitro*. If the release mechanism is similar *in vivo* and *in vitro*, a linear relation is obtained with a slope denoting the scaling factor. In the Figure 4 the *in vitro* dissolution is faster than the *in vivo*.

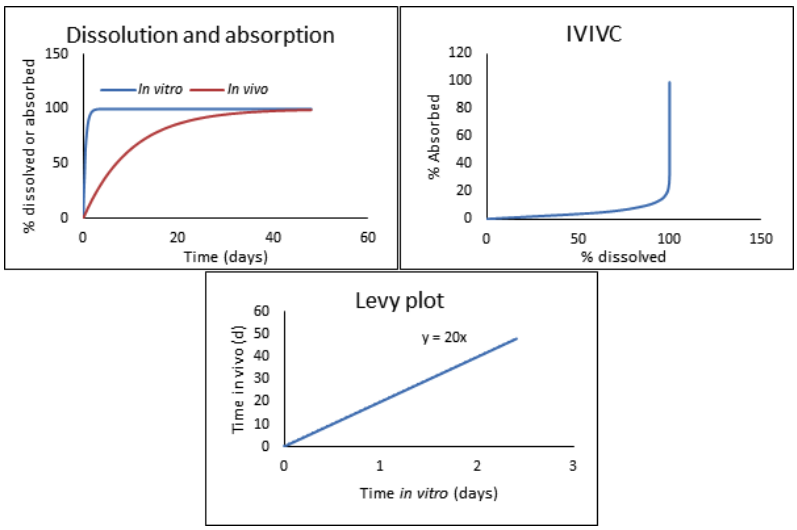

Figure 4.
Levy's plot up left comparison of dissolution and absorption curve, dissolution is more rapid, up right classical IVIVC leading to nonlinear relationship, bottom Levy's plot which will allow a 1:1 IVIVC

Full dissolution is obtained in less than 2.5 days *in vitro*, but *in vivo* release lasts for more than 48 days. The result is an IVIVC which is not linear (Figure 4 up right). The Levy plot between the *vitro* and *vivo* data is a line with a slope of 20 indicating that the *in vitro* dissolution is 20 times faster than the *in vivo* release and that the mechanisms *in vitro* and *in vivo* are similar. In case of different behaviour between *vitro* and *vivo* data (different mechanisms of release), a nonlinear Levy's plot is observed. In such a case, the first and best option is to try to find a better dissolution test, the second is to investigate *in vitro* and *in vivo* release mechanisms and justify obtained non-linearity.

A controlled release dosage form is constituted of a drug embedded in a matrix or in a reservoir. A matrix can be erodible (biodegradable) or non-erodible (based on silicon or silicone derivate, e.g. dimethylsiloxane/methylvinylsiloxane, and must be removed at the end of the release). In the first case dosage forms are constituted of some excipient which control the release of the drug, the main excipients are derived mainly from lactic and/or glycolic acid (e.g.: blend of high and low molecular weight range D-L lactide-glycolide copolymers). Controlled release dosage forms can be based either on monolithic formulations (small cylinder of 30 x 3 mm for example), substances that create a gel after injection or multiparticulate systems (applied as suspensions).

Different methodologies have been used for *in vitro* dissolution testing, and they can be classified into three main groups: sample and separate technique (using vials or USP II apparatus), dialysis sac and continuous flow-through technique (using USP IV instrument). With sample and separate technique, analysed dosage form is placed/dispersed in the dissolution medium and at predetermined time points samples are withdrawn and analysed for the drug content. Separation can be performed by ultracentrifugation or filtration. Disadvantages of this technique are difficulties in separation, insufficient agitation, risk of agglomeration (in case of microspheres). The dialysis sac technique is using semipermeable membrane; therefore the dosage form is separated from the release medium. Appropriate molecular weight cut off (MWCO) of the membrane allows diffusion of released API into the medium where the concentration is monitored. Disadvantage of this technique is potential violation of sink conditions if API release form dosage form is faster than diffusion through the membrane. The continuous flow-through technique is using USP IV apparatus and advantages of this technique are minimized agglomeration of micro-particles, media evaporation, and sample loss during separation and simple modification and replacement of media etc. [69]. On the FDA website [60] for injectable dosage forms, USP IV presents the majority of described dissolution tests (Table I). In some cases specific dissolution equipment is developed for a specific formulation. Such equipment is not described in any pharmacopeia; therefore it must be fully justified in the marketing authorization files.

Table I

FDA dissolution methods described for injectable formulations (from 60)

No.	Apparatus	Drug	Type	Method	Description	Time
1	NA	Azacitidine	Injectable Suspension		Develop a dissolution method	
2	USPIV	Betamethasone acetate/ betamethasone sodium phosphate	Injectable Suspension	IV (Flow through cell) Flow @ 8 mL/min	0.05% SLS, pH 3.0 or Develop an *in vitro* release method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 300, and 360
3	VIALS	Doxorubicin HCl	Injectable (Liposomal)		Develop a method to characterize *in vitro* release, starting at pH 6.00 ± 0.05 and at 47ºC ± 0.5ºC. Replicate for 12 dosage vials.	
4	USP IV USP II	Leuprolide acetate	Injectable (Extended Release)		Develop a dissolution method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	
5	USP IV	Medroxyprogesterone acetate	Injectable Suspension	Test 1: IV (Flow through cell), 22.6 mm cell, 13 g of 1 mm beads; Test 2: II (Paddle) Test 1: 17 mL/min; Test 2: 50 rpm	Test 1: 0.5 % SDS in water ; Test 2: 0.35 % SDS in water (provide data with both tests) Test 1: use Open Mode: Test 2: 900 mL	Test 1: 5, 10, 15, 20, 30, 40, 50, 60, 70, 80 and 90; Test 2: 5, 10, 15, 30, 60, 90, 120, 240, 360, 1440 and 2880

No.	Apparatus	Drug	Type	Method	Description	Time
6	USP IV	Methylprednisolone acetate	Injectable Suspension	IV (Flow-Through Cell-Open system)	0.55 % SDS	15, 30, 45, 60, 90 and 120
7	USP IV USP II	Naltrexone	Injectable Suspension		Phosphate buffered saline with 0.02% Tween 20 and 0.02% Sodium azide, pH 7.4 (final osmolality should be 270 ± 20 mOsm), or any other appropriate medium, at 37°C. Develop an *in vitro* release method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	
8	USP IV USP II	Octreotide Injection	Injectable (Extended Release)		Develop a dissolution method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	
9	USP II	Paliperidone Palmitate	Injectable Suspension	II (paddle) 50 rpm	0.001 M HCl containing 0.489% Polysorbate 20 at 25.0 ± 0.5°C. 900 mL	1.5, 5, 8, 10, 15, 20, 30 and 45
10	USP IV USP II	Risperidone	Injectable		Develop a dissolution method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	
11	USP IV USP II	Triamcinolone acetonide	Injectable Suspension		Develop a dissolution method using USP IV (Flow-Through Cell), and, if applicable, Apparatus II (Paddle) or any other appropriate method, for comparative evaluation by the Agency	
12	USP II	Triptorelin Pamoate	Injectable Suspension	II (Paddle) 200 rpm	Water-Methanol (95:5); Reconstitute vial in 2 mL Water for Injection, add to 500 mL medium at 37°C. 500 mL	1, 6, 12, 24, 48 and 72 hours
13	NA	Verteporfin	Injectable		Develop a method to characterize *in vitro* release.	
14	USP II	Aripiprazole	Intramuscular Suspension	II (Paddle) 50 rpm	0.25% Sodium Dodecyl Sulfate (SDS) Solution 900ml	10, 15, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min

Currently, there is no common consensus specifying release testing methodologies and development of IVIVC that could be applied to all parenteral dosage forms.

In case of microspheres, the absorption profile can show continuous delivery, or, more often, an initial burst peak followed by a second release phase. In such cases, the recommendation is to develop two different *in vitro* release methods for IVIVC establishment, as drug release mechanisms during initial and second phase are different [66]. The most common methodology for *in vitro* release testing is based on USP IV instrument, using phosphate buffer saline as medium in physiological pH range [13, 44, 45].

Development of IVIVC for liposomes is more challenging as they are designed to be quickly uptake by the RES, thus drug release kinetics might be highly dependent on the type of lipids used, surface properties, size or charge of liposomes. Researches are mostly using dialysis sac method and plasma as release medium for *in vitro* dissolution testing [50, 66].

Implants are design to release drug substance over time period of months to years. Most common *in vitro* dissolution method reported for this type of injectable dosage form are utilizing sample and separate technique (vials or flasks) in phosphate buffer saline [1, 4, 48].

Conclusions

IVIVC links *in vitro* dissolution profiles and *in vivo* data and can be used to simulate, based on *in vitro* data, the *in vivo* performance of a dosage form. Those simulations imply that the release mechanism remains unchanged between the formulations tested and the reference, being the limiting factor. Validated IVIV correlations help to understand the

formulation behaviour and mechanism establish dissolution specification limits and allow the use of *in vitro* data as a surrogate of *in vivo* studies mainly in case of level A correlation.

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