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**ORIGINAL ARTICLE**

# Selection of the most suitable dissolution method for an extended release formulation based on IVIVC level A obtained on cynomolgus monkey

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**Abstract**

*Objective*: The purpose of this study is primarily to identify the most suitable in vitro dissolution method(s) for their ability to predict the in vivo performance of extended release prototype tablet formulations designed for a new chemical entity, Biopharmaceutic Classification System class II drug, weak base, based on the data collected in cynomolgus monkey. *Materials and methods*: Different types of buffer at different pH were selected as dissolution medium resulting in a broad variety of release patterns (slow to fast). The in vivo and in vitro data were put in relation. *Results*: As a consequence of the discrimination between both tested formulations, the in vitro–in vivo correlation (IVIVC) qualities and shapes changed significantly. The obtained level A showed that the simple HCl medium was superior to biorelevant media and media containing surfactant when investigating IVIVCs in cynomolgus monkey. In addition, the results of dissolution in HCl suggested rather a diffusion mechanism of the extended release matrix formulation as the main factor of the release. *Conclusion*: Adjusting dissolution testing conditions to match the behavior of the formulations in vitro with that in vivo by taking into account the properties of the drug and the formulation is a straightforward and useful approach in identifying a predictive method in the development of the IVIVC. These investigations will definitely help by derisking of new formulations as well as by rating changes in existing formulations with regard to their impact on bioavailability before entry into human.

**Key words:** *BCS class II; dissolution; extended release; IVIVC*

## Introduction

A common challenge of all pharmaceutical companies is the development of new drugs, as fast as possible, to cover unmet medical needs and at the same time to ensure safety and efficacy. Many strategies exist and among them in vitro dissolution, animal experiment, and in vitro–in vivo correlation (IVIVC) can be used early in the development phase to minimize the risk before administration into man, to address the impact of changes in existing formulations with regard to bioavailability (BA), and to improve the development strategy leading to a faster time to market.

In this study, this approach was used to select an extended release (ER) formulation of a new chemical entity, weak base classified as a poorly soluble and highly permeable drug [Biopharmaceutic Classification System (BCS) class II[1]]. This drug, after oral administration of an immediate release (IR) capsule in human, exhibited side effects hypothetically because of high plasma concentration. To address this high concentration ($C_{max}$), prototype formulations of ER hydrophilic matrix tablets were developed and optimized using dissolution techniques to sustain $T_{max}$ and lower $C_{max}$. During this development, dissolution data were generated to assess the formulations performance throughout the optimization process. However, at this stage, the discriminative power and the effectiveness of the dissolution method as a predictive tool to derisk human in vivo study are unknown. Therefore, the development of

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the initial dissolution method for this poorly soluble compound included the assessment of relevant physical and chemical properties of the drug as well as the key factors of the drug product and formulation. The two optimal formulations, with regard to the dissolution performance observed in the simplest media achieving sink condition, were tested in animals before any new administration to humans to demonstrate its technical feasibility and efficacy. A systematic screening of various classical dissolution media keeping the apparatus and condition similar was realized to better understand the behavior of these two formulations, and a relationship between in vitro and in vivo animal data was assessed.

The combination of these tools (dissolution, animal data, and correlation as well as some weakness of in silico data) to develop and select the most suitable in vitro method is discussed in this article as a smart development tool to speed up the realization of new formulations and to ensure the best performance during future human trials.

## Materials and methods

### *Materials*

Egg lecithin (E PC S, purity >96%) was obtained from Lipoid (Ludwigshafen, Germany), and sodium taurocholate (NaTC, 97% pure) used was received from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy.

Phosphate buffer, sodium chloride, 37% hydrochloric acid (fuming), 85% ortho phosphoric acid, ethanol (99.9%) as well as high performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Tris buffer was obtained from Applichem (Darmstadt, Germany) and water was obtained from Milli-Q (Millipore, Milford, MA, USA) water purification system.

The various surfactants, namely, sodium lauryl sulfate (SDS), hexadecyl-trimethyl-ammonium bromide (CTAB), and polysorbate 80 (Tween 80), were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

### *Model compound and solid formulations*

The poorly soluble new chemical entity Roche compound (RO-X) is a small molecule, a weak base used in the form of hemisulfate salt. Only one polymorph form is known and is stable in water. Its main physicochemical properties are ClogP of 3.7 with p$K_a$ 4.6. The experimental dose was set at 1 mg.

Experimental IR capsule formulation and ER tablet formulations were supplied by Roche Pharmaceutical Research Department. The ER formulations were produced by wet granulation using the same batch of active pharmaceutical ingredient (API). Different amounts of hydroxypropylmethylcellulose (HPMC) were adjusted to decrease the $C_{max}$ having a target of 85% release in vitro within 4 or 8 hours, respectively. The IR reference formulation exhibits a complete release within 0.5 hour. All formulations were designed and homotetically adjusted for administration in monkey.

### *Methods*

#### Dissolution media preparation

Various media were tested from pH 1.1 to 6.5. Compendial media were prepared according to the US Pharmacopeia. Phosphate buffers with pH 4.0 and 6.0 were prepared from 0.05 M sodium dihydrogen phosphate. pH was adjusted using 0.2 M sodium hydroxide (NaOH) or phosphoric acid. The amount of surfactant was added accordingly. Because of the low solubility, addition of three types of surfactant (anionic, cationic, and nonionic) was tested at pH 6.0 to have (i) a pH close to neutrality, (ii) a pH more in accordance with gastrointestinal (GI) tract pH[2,3], (iii) a similar effectiveness of all the surfactants, and (iv) a reasonable stability of the solutions after filtration. Biorelevant media, fasting state simulated intestinal fluid (FaSSIF) and fed state simulated intestinal fluid (FeSSIF), were prepared according to current procedures[4]. Overall, the media were stable and present as a single phase. Two steps were included in the dissolution method: step 1 was handled in the selection of the best formulations to be tested in vivo on monkey and corresponded to acidic media alone; and step 2, after having the in vivo results, corresponded to the screening of various dissolution methods to seek for the most pertinent IVIVC.

#### Solubility studies

The solubility of the RO-X was assessed in each dissolution medium. Duplicate samples were incubated with an excess of compound in a 10 mL volumetric flask at 37°C with constant rotation. Samples were collected after 4 and 24 hours, filtrated, and subsequently diluted with the HPLC mobile phase. The dissolved quantity was measured with a validated HPLC–ultraviolet (UV) detection method. The solubility studies were carried out with the same batch of API that was used for manufacturing of the tablets.

#### Dissolution studies

The dissolution profiles of the ER formulations were examined in different media, using a Sotax AT7 smart apparatus equipped with automated sampling (Sotax, Allschwil, Switzerland). In all cases, paddle speed and temperature were set at 50 rpm and 37°C, respectively, with $n = 3$ units. Because of the low dose of the tablets (1 mg) and sensitivity of the analytical method, the dissolution volume was held constant at 500 mL (minimal volume insuring a homogeneity and reproducibility of

the media). Ten 1 mL samples were withdrawn at predefined time intervals up to 8 hours from each vessel and not replaced. Test solution (20 mL) was pumped through the circuit before each sampling time to prerinse the sampling lines and filters. Sampling and filtration were automated and dissolution samples were directly filtered and subsequently measured by a validated HPLC–UV detection method.

**In vivo bioavailability assessment**

A pilot study based on a single dose, three arms simple study comparing the two ER tablet formulations (target 4 and 8 hours release) with the IR formulation (reference capsule) was performed on three cynomolgus monkeys in fasting conditions. Because of the animal's ethical limitations, only limited number of samples could be withdrawn, and particular attention was given to the early time points. The samples were collected at predefined time intervals (0, 0.5, 1, 2, 3, 4, 7, 24, 32 hours) and measured by a validated HPLC–MS method.

**In vivo–in vitro correlation**

For the in vivo data, in addition to the classical BA parameters $C_{max}$, $T_{max}$, and AUC, the percentage of fraction of drug absorbed (%FD) was determined by deconvolution using the Wagner–Nelson (WN) method[5,6]. In vitro the percentage of drug dissolved (%D) was obtained from the dissolution. Various approaches of relationship between in vitro and in vivo data were examined based either on values (IVIVC[7,8]) or on rank (IVIVR[9,10]). The in vivo and in vitro data were put in relation using a point-to-point relationship between the in vitro dissolution and the in vivo input of the drug (IVIVC level A). Linear regressions were primarily sought. In case of faster dissolution than input, a scaling factor from linear Levy plots[11] or from nonlinear scaling[12] was discussed.

Model predictability was estimated internally by comparison of prediction errors for pharmacokinetic parameters, $C_{max}$, $T_{max}$, and AUC, derived from mean observed and predicted in vivo data obtained by using the inverse of WN method[13]. For a reasonable IVIVC, regulatory guidelines state prediction errors for $C_{max}$ and AUC should not exceed 10%[14,15]. All calculations were done using Microsoft Excel.

## Results

### *Solubility studies*

The equilibrium solubility at 37°C over the physiological pH range using classical dissolution media after 4 and 24 hours is presented in Table 1.

The molecule exhibits a typical pH depending solubility profile of a weak base with a low solubility at high pH. The solubility in the biorelevant media (FaSSIF and FeSSIF) was estimated to be 12.5 mg/500 mL and 60 mg/500 mL, respectively, denoting an improvement of roughly 30 times at pH 6.5 in FaSSIF and of around 140 times for FeSSIF compared to the classical pH 6.0 phosphate buffer. Addition of chemical surfactants of either nature between 0.5% and 1% in dissolution media at pH 6.0 led to solubility estimated to be greater than 7.5 mg/500 mL (increase of solubility more than 18 times). Acid pH, biorelevant media, and media with adjunction of surfactant exhibit sink conditions for the 1 mg dose, which is not the case for pure phosphate buffer pH 6.0. Overall, no shifts in pH or precipitation were observed during equilibrium solubility determinations.

**Table 1.** Solubility of RO-X in the various dissolution media over the physiological pH after 4 and 24 hours at 37°C.

| Medium | pH | mg/mL After 4 hours | mg/mL After 24 hours |
|---|---|---|---|
| HCl 0.1 N | 1.1 | 13 | 13 |
| Phosphate 0.05 M | 4.0 | 1.4 | 1.4 |
| Phosphate 0.05 M | 6.0 | 0.0008 | 0.0008 |
| FeSSIF | 5.0 | – | 0.12 |
| FaSSIF | 6.5 | – | 0.025 |
| Phosphate 0.05 M + 0.5% SDS | 6.0 | >7.5 | >7.5 |
| Phosphate 0.05 M + 1% CTAB | 6.0 | >7.5 | >7.5 |
| Phosphate 0.05 M + 1% Tween | 6.0 | >7.5 | >7.5 |

### *Paddle dissolution studies*

Six different ER tablets with different amounts of HPMC (formulations 1–6) were first measured by dissolution for prescreening using HCl 0.1 N (step 1). The IR formulation is presented on those curves as a reference in Figure 1. Formulations 2 and 4 exhibited profiles closer to the targets and were selected. Both tablets were further tested using the seven dissolution media (see Tables 2–4). The

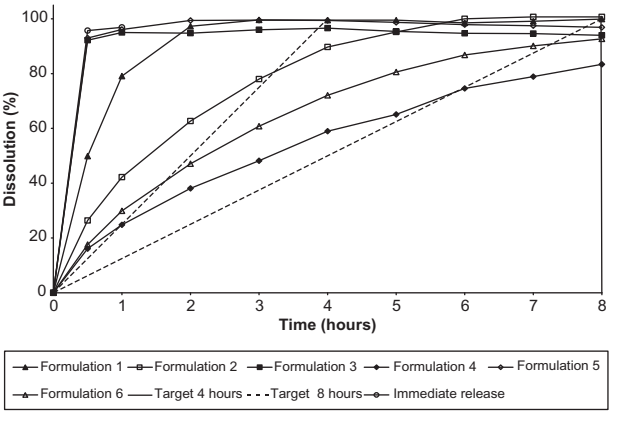


**Figure 1.** Dissolution profiles of different ER tablets versus IR formulation in HCl 0.1 N.

**Table 2.** Overview of the obtained $R^2$ after IVIVC level A attempts.

| $R^2$ | HCl 0.1 N | pH 6.0 | FaSSIF | FeSSIF | pH 6.0 + CTAB 1% | pH 6.0 + Tween 80 1% | pH 6.0 + SDS 0.5% |
|---|---|---|---|---|---|---|---|
| Form. No. 2 | 0.97 | 0.96 | 0.96 | 0.93 | 0.95 | 0.73 (0.99)[a] | 0.98 |
| Form. No. 4 | 1.00 | 0.99 | 0.96 | 0.99 | 0.94 | 0.89 | 0.90 |
| Form. Nos. 2 + 4 | 0.90 | 0.67 | 0.87 | 0.63 | 0.64 | 0.48 | 0.24 |

[a]Value without burst effect after 4 hours $R^2 = 0.99$.

**Table 3.** Overview of the obtained slope after IVIVC level A attempts.

| Slope | HCl 0.1 N | pH 6.0 | FaSSIF | FeSSIF | pH 6.0 + CTAB 1% | pH 6.0 + Tween 80 1% | pH 6.0 + SDS 0.5% |
|---|---|---|---|---|---|---|---|
| Form. No. 2 | 1.051 | 1.411 | 5.967 | 1.136 | 1.449 | 1.125 | 1.651 |
| Form. No. 4 | 0.960 | 1.506 | 6.215 | 7.431 | 2.770 | 4.819 | 5.081 |
| Form. Nos. 2 + 4 | 0.885 | 0.802 | 5.135 | 0.958 | 0.997 | 0.879 | 0.720 |

**Table 4.** Overview of the obtained intercept after IVIVC level A attempts.

| Intercept | HCl 0.1 N | pH 6.0 | FaSSIF | FeSSIF | pH 6.0 + CTAB 1% | pH 6.0 + Tween 80 1% | pH 6.0 + SDS 0.5% |
|---|---|---|---|---|---|---|---|
| Form. No. 2 | −33.32 | −47.74 | −23.55 | 2.15 | −15.06 | 2.61 | −84.32 |
| Form. No. 4 | −13.47 | −10.52 | −7.81 | −7.96 | 5.72 | 9.45 | 3.40 |
| Form. Nos. 2 + 4 | −16.28 | −1.63 | 9.02 | +13.40 | 10.05 | 16.54 | 14.34 |

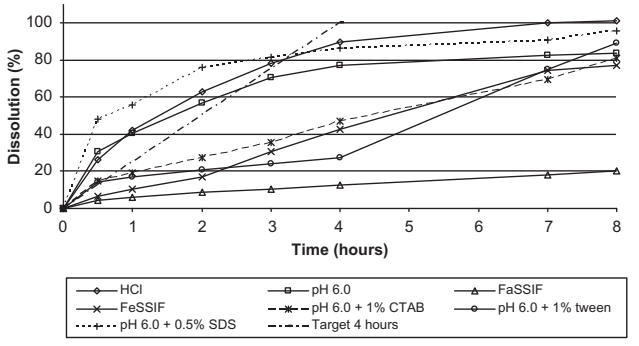


**Figure 2.** Dissolution profiles of formulation 2 (ER4H) within all tested media.

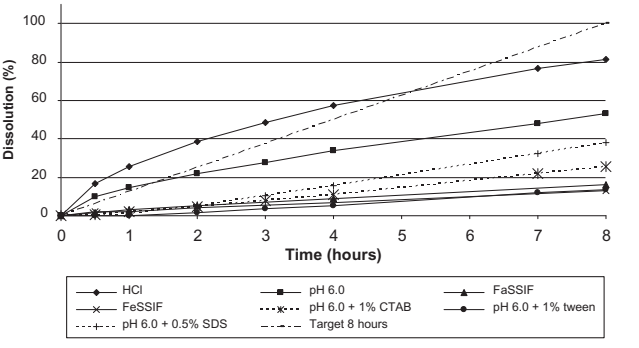


**Figure 3.** Dissolution profiles of formulation 4 (ER8H) within all tested media.

results of the dissolution tests performed with various media (step 2) on formulations 2 and 4 are presented in Figures 2 and 3.

Dissolution of both chosen formulations (2 and 4) exhibited the expected rank order, irrespective of which medium was employed. Standard deviations were observed in the range of 3–5%. No coning or mounting was observed during the dissolution tests.

### *In vivo data*

The in vivo cynomolgus monkey results are presented in Figure 4 and in Table 5 for pharmacokinetic parameters (PK) parameters.

The in vivo release data demonstrated that compared to the reference (IR capsule), a lower $C_{max}$ and a prolonged $T_{max}$ can be observed with the ER tablets indicating a slower absorption and an impact of the composition of the tablets on the global performance. The two slow release formulations exhibited a monophasic decline indicating an apparent one-compartment model. In this case, the Wagner[6] method can be used for deconvolution and the results are presented in Figure 5 up to 7 hours (100% of absorption being reached later). Figure 6 presented the percent remaining to be absorbed denoting an apparent first-order kinetic for both ER formulations.

### *In vitro–in vivo correlation*

The basic comparison of the dissolution data and the in vivo data indicated a correct ranking of both formulations independently of the media used.

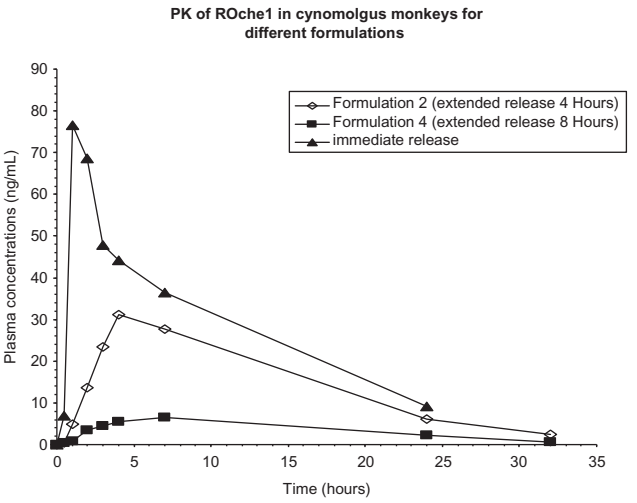


**Figure 4.** PK mean profiles of RO-X in cynomolgus monkeys for the three tested formulations (ER formulations 2 and 4 versus reference IR formulation).

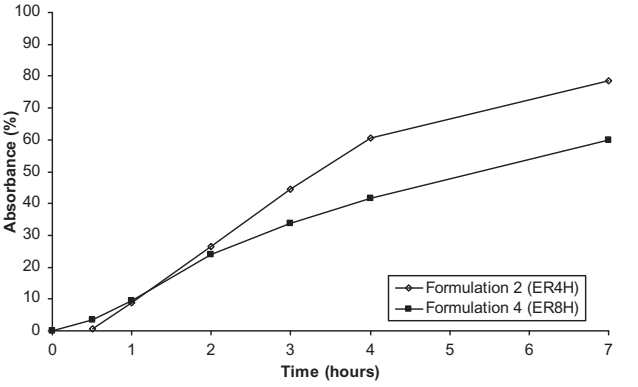


**Figure 5.** Percentage of fraction absorbed as function of time according to Wagner–Nelson.

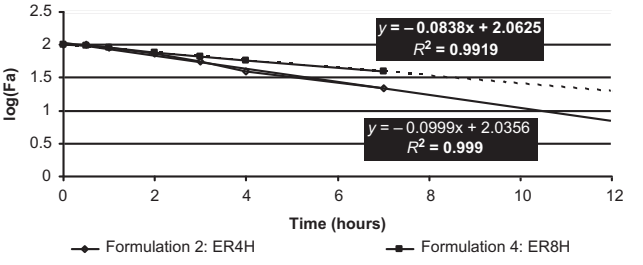


**Figure 6.** Logarithm scale of Wagner–Nelson.

The various level A IVIVCs attempted with the different media tested are presented in Figure 7a–g and in Tables 2–4.

No IVIVC of level C or IVIVR was further investigated as the level A IVIVC can be established.

## Discussion

The IR formulation exhibited a fast absorption ($T_{max}$ = 1 hour) and a high peak followed by a biphasic decline denoting a two-compartment model. This biphasic decline was not observed for both ER formulations confirming, as they behaved as a one-compartment model, the possibility to use WN equation.

For ER formulations, the observed $T_{max}$ are close to the forecasted time of release. It has to be noted that the lack of sampling time points between 7 and 24 hours, because of limited blood volumes permitted in monkey, might underestimate the $T_{max}$ value of formulation 4 as well as $C_{max}$ and AUC. AUC decreased when absorption was slower (lower $C_{max}$ and increased $T_{max}$). The decrease of AUC was, as a mean, lower than the $C_{max}$ decrease but of a similar magnitude. For formulations 2 and 4, the diminution of $C_{max}$ was, respectively, 48% and 91% and of 38% and 85% for AUC. The GI transit time of the monkey being shorter than man[16], incomplete absorption because of the slow release was anticipated, in particular for formulation 4. Because of the longer residence time in man, the decrease in exposure is expected to be lower. If high initial $C_{max}$ values are related to the observed clinical side effects, then the modified release formulation meets the target, and technical feasibility and efficacy are validated.

Seven different dissolution media at various pHs from HCl 0.1 N pH 1.1 to phosphate buffers pH 6.0, from a FaSSIF without lipid components of pH 6.5 to a FeSSIF of pH 5.0, were employed in the dissolution USP 2 apparatus. All the media studied were capable to some extent of differentiating between both formulations and the expected rank order was found for formulations 2

**Table 5.** Arithmetic means (±SD) of PK parameters of the RO-X on cynomolgus monkeys for the three tested formulations (ER formulations 2 and 4 versus reference IR formulation).

| Parameter | Unit | Formulation 2 ER4H | Formulation 4 ER8H | IR formulation |
|---|---|---|---|---|
| $C_{max}$ | ng/mL | 36.4 (±17.6) | 6.47 (±5.2) | 79.1 (±9.0) |
| $T_{max}$[a] | hours | 4 | 7 | 1 |
| AUC(0–inf) | (ng·h)/mL | 464 (±145) | 114 (±80) | 847 (±218) |

[a]Median.

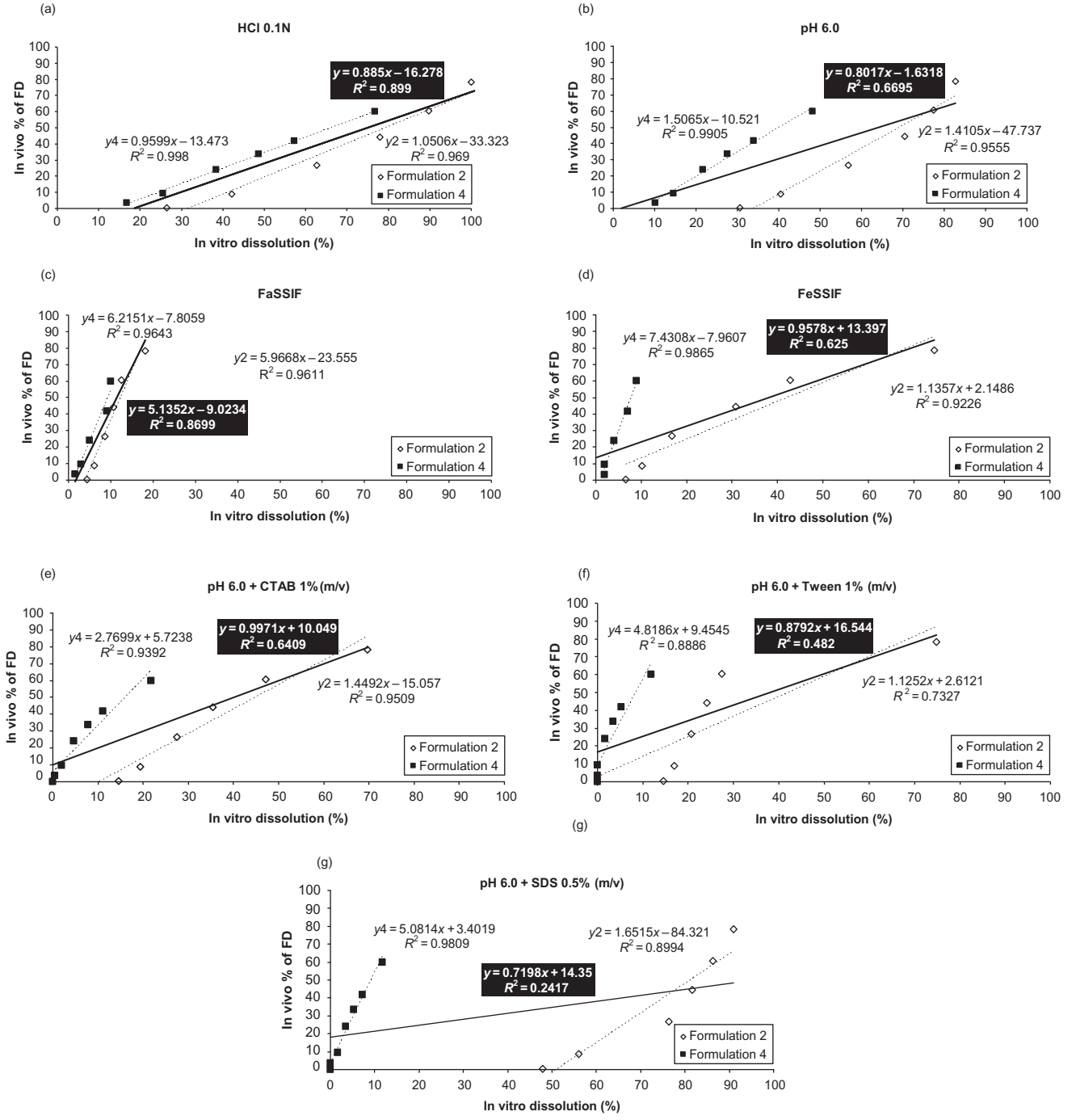


**Figure 7.** IVIVC attempt for both formulations 2 and 4 in different media: (a) HCl 0.1 N, (b) buffer pH 6.0, (c) FaSSIF, (d) FeSSIF, (e) buffer pH 6.0 with CTAB 1% (m/v), (f) buffer pH 6.0 with Tween 1% (m/v), (g) buffer pH 6.0 with SDS 0.5% (m/v). Full line indicates the overall correlation for formulations 2 and 4, open symbols indicate formulation 2 and full symbols formulation 4. Corresponding equations are identified with *y*2 and *y*4, and *y* for overall correlations.

and 4 (ER4H and ER8H), respectively. Despite the fact that based on the measured solubility, the entire drug should be freely dissolved into the volume tested, huge differences in dissolution rate as well as very poor recovery were found for some profiles. Profiles obtained in HCl and phosphate pH 6.0 exhibited the highest performance for both formulations. The dissolution profiles are faster than all the others in HCl 0.1 N (pH 1.1). Because HPMC is a polymer with a release behavior independent of the pH[17] and because sink condition

can be easily reached at pH 1.1 (see Table 1), the phenomenon observed in dissolution was the sole reflect of the influence of the excipients on the release of the drug. The improvement of the apparent solubility at pH 6.0 by the addition of surfactant of either nature did not improve the dissolution and led to worst results than those observed at pH 6.0. Surprisingly good dissolution up to 80% was observed in phosphate buffer at pH 6.0 (at pH 6.0 sink condition was not reached). The good pH 6.0 results were not expected because the drug is a weak base with p$K_a$ of 4.6 and solubility decreases with increasing pH. The increase in solubility between FaSSIF and FeSSIF was only reflected slightly in the dissolution of formulation 2. By contrast no significant difference can be observed for formulation 4. The release in FaSSIF medium is much slower than in any of the other media examined. This is not expected in view of the high solubility measured in this medium but can be explained to some extent by the low buffer capacity of such a medium as already reported in the literature[18]. It appears to be clear that using biorelevant media and media containing surfactant, the release mechanisms which generally control hydrophilic matrix tablets by diffusion, swelling, and/or erosion[17,19,20] are strongly impacted depending on the HPMC ratio used in the ER formulations. Therefore, the observed in vitro performances seem to be controlled not by the solubility of the API but rather by the release mechanism. The RO-X molecule behaves in this environment presumably more likely as a BCS class I than like a classical weak base, BCS class II. The dissolution limiting step is controlled solely by the formulation[21,22].

By applying IVIVC, in vitro release profiles were compared to the corresponding in vivo input profiles. For most of the media a linear response ($R^2$ close to 1) for solely formulation 2 or 4 (ER4H or ER8H) can be achieved (see Table 2). Depending on the medium used, similar slopes for ER4H or ER8H can be observed (see Table 3). The use of various in vitro working conditions improved differentiation between formulations but did not necessarily lead to an acceptable and useful correlation, with in vivo absorption rates having both formulations 2 and 4 fitted simultaneously. The systematic shift of the correlation shape underlines the sensitivity of the IVIV relationship to medium composition and release mechanism.

Because the release mechanism changes depending on the medium used (but should be similar within the same medium for both formulations), the observed difference in slope can highlight that the dissolution rate is different between in vivo and in vitro, which indicates rather a nonsuitable dissolution.

In case of strong positive intercept (Table 4), the relationship is considered as not of a good quality as a certain percent is supposed to be absorbed when no part is dissolved in vitro (e.g., in medium with surfactant). Similarly, a slope markedly greater than 1 could indicate that a great part (even 100%) is absorbed when only a small fraction is dissolved (e.g., FaSSIF). In case of a slope lower than 1, a time scaling factor (e.g., Levy plotting) could be investigated but as the low percentage dissolved that is observed should correlate to a high quantity absorbed, the conclusion would be that the dissolution test did not adequately reflect the in vivo behavior. Thus, it is obvious that the observed relationships denote that some media are not adequate to perform IVIVC. The correlations resulting from the media containing surfactant are the most weak and might be due to interaction between the surfactants and the excipients leading to similar in vitro dissolutions, hiding differences in release, even if differences between the release rates existed in vivo. Both ER formulations exhibited similar apparent absorption mechanism (see Figure 6); the first choice for the level A correlation focused on the media where each tested formulation resulted as well in a similar behavior with regard to release rate. The aim was to obtain the simplest model possible. With this consideration in mind, the only apparent IVIVC that showed similar drug release mechanism and that was linear and resulted in similar slope for both ER variants was obtained using FaSSIF, pH 6.0 buffer, and HCl 0.1 N. For these three media, release did not start at a similar time; formulation 2 is faster than 4, which is in line with the expectations. However for FaSSIF, the slope is largely greater than 1 indicating that for a small amount dissolved (e.g., only 20% dissolved after 7 hours for formulation 2), a large amount was absorbed leading to the conclusion that this medium is not optimal. For pH 6.0 buffer, a great difference in intercept was observed for formulation 2 compared to formulation 4, leading to a poor overall coefficient of correlation ($R^2$ = 0.67). The negative intercept was always longer for formulation 2 compared to formulation 4 (Table 4), indicating that in vitro the dissolution was slightly faster than the absorption for formulation 2 compared to 4. The best level A correlation observed was obtained using HCl 0.1 N. The overall linear regression yielded a regression coefficient $R^2$ of 0.90.

By applying the IVIVC equation obtained with HCl, a prediction according to the inverse of WN method[13] and based on the observed in vitro dissolution was tried and the results are presented in Figure 8.

The prediction resulted in an error of +13% and −7% for $C_{max}$ for ER4H and ER8H, respectively, and a negligible error for AUC and $T_{max}$ (less than 1%) denoting a rather good predictability. Based on this correlation, dissolution in HCl 0.1 N medium can adequately support the development and improvement of further formulation.

Among the methods in the literature to ascertain the kinetic modeling of drug release, for example, zero-order,

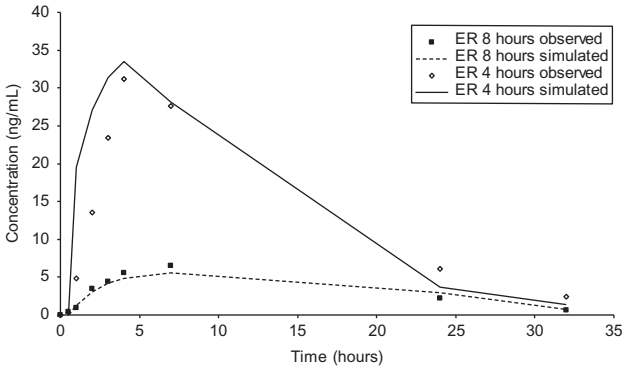


**Figure 8.** Prediction of absorbance profiles based on HCl 0.1 N dissolution data and IVIVC.

first-order[23], Higuchi[24], Hixon–Crowell[25], and Weibull models[26,27], the exponential equation of Korsemeyer and Peppas[28] and that of Harland[29–31] are used to describe the drug release behavior from polymeric systems. These models are generally used to analyze the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomenon could be involved. As the equation of Korsemeyer and Peppas can only be used to fit 60% release, the best fit was obtained by using the equation of Harland et al. Thus, the dissolution profiles can be fitted to Equation (1):

$$\frac{M_t}{M_\infty} = A\sqrt{t} + Bt \qquad (1).$$

In the above equation, $A$ and $B$ are diffusion and erosion terms. When $A > B$, the diffusion factor prevails in the release system. When $A <$ B, erosion predominates. If $A = B$, the release mechanism includes both diffusion and erosion equally[32].

The dissolution profiles in HCl fitted by Equation (1) suggested clearly an apparent diffusion mechanism associated to the in vitro release for both formulations (ER4H $A = 0.38$, $B = 0$; and ER8H $A = 0.25$, $B = 0.02$) and confirmed that both formulations behave similarly in this medium. Controlling of the diffusion behavior can then be an additional monitoring parameter to support new formulations or change during further development.

In comparison to more complex approaches using simulated intestinal fluids as often seen in literature reports[33–35], the use of this simple dissolution method is advantageous because it has the potential to serve both as a robust quality control method and as a biorelevant method with discrimination power.

Attention has to be paid to the fact that the present observed correlation is valid only for an apparent one-compartment model in vivo absorption using a hydrophilic matrix. Overestimation of these criteria can lead to misinterpretation even if the mathematical correlation seems to suggest a reliable prediction of the in vivo performance[36,37].

Additionally, it is important at this stage of the development to note that this animal approach using IVIVC has a number of limitations, the major one being the GI capacity and transit time of monkey compared to humans. Monkeys are closer to humans in terms of evolutionary development than all other commonly used laboratory animals such as rodents or dog[38]. As a model for oral drug absorption, cynomolgus monkeys can be used to address intra- and interindividual physiological variability[39]. Apart from species differences in intestinal physiology, erroneous assumptions can also be made with regard to scaling of dosage. In our case, the drug was tested in animals as side effects were observed in humans and a new in vivo study in humans could not be proposed before the safety of the drug was assessed in animals. Rodents cannot be used because of the size of the tablet; using dogs was not possible because of constriction force of their stomach that is known to destroy HPMC matrix[40]; and therefore monkey was the only practicable species. The large variability observed between animals implied difficulties in predicting small variations and limiting the discriminative power the IVIVC linked to minor changes of the formulation. However, the aim of this work was to be able to discriminate formulations with a large release difference to insure the best selection before human study and to check which dissolution test was the most appropriate based on the mean curve which is an unbiased estimation.

In silico estimation was evaluated as well but it exhibited some limitations. Nowadays, several computational simulation programs are available[41–43] and are offering specific modules (e.g., IVIVC Toolkit). However, computational simulation is not always accurate because it is based on many assumptions like numeric integration of the Noyes–Whitney equation for the dissolution or the membrane permeation equation. Incorporation in the simulation of factors like the relationship between disintegration, dissolution, and erosion–diffusion mechanism or furthermore the increase of solubility because of addition of surfactant but leading to slower dissolution as observed within this investigation cannot be easily set up using the current version of these software[44,45]. In most of the cases, a model can only be adequately set up after a first comparison with real experimental data and some adjustments[46]. Thus, until these criteria can be adequately modeled, generation of the appropriate in vitro data to try to approximate the in vivo behavior and use as input to establish a correlation will remain more accurate. However, the data generated within this study

to demonstrate its technical feasibility for the ER formulation enable now a better basis for further in silico projection, in particular for simulating human behavior based on animal data.

For drugs like RO-X associated as low dose with hydrophilic matrixes, first attempts can be done in classical acidic media (pH 1.1–2.5) and neutral media (pH 5.5–7.0). In case of similar and conclusive results between the two dissolution tests, animal models can be used to confirm the ranking of the formulations and the expected results. In this case an IVIVC/IVIVR investigation can be much easily set up. As general rule for IVIVC investigation, the correlation is more realistic if the release mechanism and rate observed in vivo are reflected in vitro. In vitro working condition can be adapted consequently in case of poor relationship. The information likely to be gained is worthwhile and in this way, efforts to achieve a correlation facilitate formulation screening at the early development stage by the better understanding of key parameters that are likely to impact the drug product performance. Additionally, the use of IVIVC based on animal species in early stage potentially reduce the number of animal studies, that are typically done for formulation screening, that being in line with current recommendations. Anyhow these investigations provide valuable information to better streamline the drug development process and offer help in evaluating manufacturing process parameters at later stages.

## Conclusion

The in vitro release from the developed formulations was found to be independent of solubility and pH but dependent mainly on the composition of the dissolution media. This investigation shows that the simple HCl medium was superior to a biorelevant medium and medium containing surfactant when investigating the drug in cynomolgus monkey and establishing IVIVC. The results of the dissolution in HCl have helped to identify the diffusion mechanism, from a HPMC-based ER formulation, as the apparent key parameter of the release mechanism.

The significance of this study may be applicable to other ionizable weak base drugs with high permeability. For this BCS class II compound (weak base, low drug load), the dissolution rate of this ER form is limited not by the solubility over GI tract pH but mostly by the release mechanism. During early drug development, it is extremely useful to have a predictive in vitro dissolution test that correlates with in vivo absorption. Such a test helps in the screening of new formulations as well as evaluating changes in existing formulations with regard to their impact on BA. In this case, further research has to be conducted to confirm this outcome in man. In conclusion, adjusting dissolution testing conditions to match the behavior of the formulations in vitro with that in vivo is a simple and useful approach in identifying a predictive method for the development of IVIVC and allows clearly decreasing the risk before first entry into human.

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## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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