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RESEARCH PAPER

In Vivo Bioequivalence and *In Vitro* Similarity Factor (f2) for Dissolution Profile Comparisons of Extended Release Formulations: How and When Do They Match?

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ABSTRACT

Purpose To investigate how likely two extended release formulations are to be bioequivalent when they demonstrate f2 similarity.

Method Dissolution profiles were simulated using the Weibull model and varying model parameters around those of a reference profile. The f2 values were calculated for the comparisons of each simulation with the reference profile. The *in vivo* inputs obtained from an *in vitro-in vivo* correlation model were convolved with a unit impulse response function. The AUC, Cmax, and Tmax from each simulated *in vivo* concentration profile were compared to the reference profile. The AUCR (AUC ratio) and CmaxR (Cmax ratio) were determined. The consistency between f2 and bioequivalence was investigated.

Results The relationships between AUCR, CmaxR, f2 and the Weibull model parameters demonstrate that the bioequivalence regions enclosed by the contour lines of 80% and 125% of AUCR and CmaxR were generally close to the regions enclosed by the f2 = 50 contour line, but did not exactly match, especially when Dmax and B deviated from the reference values.

Conclusions When f2 is used for *in vitro* dissolution profile comparison, the completeness of the dissolution profiles should not differ more than 10%, and the shapes of the dissolution profiles should not be significantly different.

KEY WORDS bioequivalence · dissolution · f2 similarity factor · IVIVC · modeling and simulation

INTRODUCTION

Establishment of bioequivalence is necessary for formulation and/or manufacturing changes occurring during the drug development and post-approval stages. Bioequivalence is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (1). In bioequivalence studies, the systemic exposure profile of a test drug product is compared to that of a reference drug product. If the 90% confidence intervals of the ratios of the exposures (mainly AUC and Cmax) between the test formulation and the reference formulation fall within 80–125%, the test formulation can be considered bioequivalent to the reference. Bioequivalence studies can be largely due to variability-driven power calculations and can thus be very costly. Therefore, it is desirable to avoid such studies if possible. Under certain circumstances, bioequivalence could be assured by *in vitro* dissolution profile comparison.

Dissolution profile comparisons have extensive applications throughout the product development process. When composition, manufacturing site, scale of manufacture, manufacturing process and/or equipment have changed within defined limits, dissolution profile comparison can be used to establish the similarity between the formulations

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pre- and post-changes. The FDA has issued guidance documents for both immediate-release (IR) formulations (2,3) and modified-release (MR) formulations (4,5). These documents indicate the type of data that are accepted in support of post-approval changes to the formulation, and their aim is to reduce the regulatory burden by decreasing both the number of manufacturing changes that require FDA prior approval and the number of bioequivalence studies necessary to support these changes. Therefore, for certain formulation changes, establishing similarity between dissolution profiles for the test and the reference formulation batches in several media is considered sufficient justification. The assumption is that the test product is bioequivalent to the reference product if *in vitro* similarity is established.

As a result of the potential to obtain a waiver for *in vivo* bioavailability and bioequivalence studies with an *in vitro* method, interest among pharmaceutical scientists has focused on the methodology used to compare dissolution profile data. A dissolution profile is defined as the measured fraction (or percentage) of the labeled amount of drug that is released from a dosage unit (such as tablet or capsule) at a number of predetermined time points when tested in a dissolution apparatus, such as the US Pharmacopeia (USP) I or II dissolution systems (6).

The mathematical methods for the comparison of dissolution profiles are described in the literature. One of these was published by Moore and Flanner (7). They described a 'similarity factor' also known as the f2 equation, which is a logarithmic transformation of the average of the squared vertical distances between the test and reference mean dissolution values at each dissolution time point as shown in Eq. 1:

$$f2 = 50 \times \mathrm{Log}\left\{\left[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (1)$$

where Log=logarithm to base 10, n=number of sampling time points, ∑=summation over all time points, R_t and T_t are the reference and test dissolution values (mean of at least 12 dosage units) at time point t.

The value of f2 is 100 when the test and reference mean profiles are identical. The maximum distance between mean dissolution profiles at any time point cannot exceed 100%, in which case the value of f2 will be close to zero. An average difference of 10% at all measured time points results in a f2 value of 50. Therefore, it is believed that values of f2 between 50 and 100 ensure sameness or equivalence of the two dissolution profiles and, thus, the performance of the two products (1–4,8).

The f2 equation is the most popular method used to compare dissolution profile data, given that it is recommended for use in a number of FDA guidance documents (1–5,8). The major advantages of the f2 equation are that it is easy to compute and it provides a single number to describe the comparison of dissolution profile data. However, there are disadvantages (9–12). The f2 equation does not take into account the variability or correlation structure in the data. Also, the values of f2 are sensitive to the number of dissolution time points used. Furthermore, the basis for the criteria to determine the similarity between dissolution profiles is unclear. This last point touches on an important issue in the dissolution profile comparison—the practical significance of differences between mean dissolution profiles. That is, how large can the difference between mean dissolution profiles be before the differences are likely to impact on *in vivo* performance?

Knowing how consistent f2 similarity is with the criteria for bioequivalence is important for assuring similarity in product performance. It is necessary to address the following two questions: 1) how likely are the two products determined to be similar *in vitro* not to be bioequivalent (false positive) and 2) how likely are the two products determined to be dissimilar *in vitro* to be bioequivalent (false negative). The goal of this study was to investigate the consistency between the *in vitro* dissolution profile comparisons using an f2 matrix and *in vivo* bioequivalence using the 80–125% criteria for an extended release formulation. We utilized a simulation approach to examine several potential scenarios to get a general picture about this issue.

MATERIALS AND METHODS

Dissolution Model and Dissolution Profile Generations

A Weibull model was used to generate the *in vitro* dissolution profiles as shown in Eq. 2.

$$\%\,\mathrm{Dissolved} = D_{max} \times \left(1 - e^{-\left(\frac{\mathrm{Time}}{\mathrm{MDT}}\right)^B}\right) \quad (2)$$

where Dmax is the maximum dissolved (% of the labeled amount), MDT is the mean dissolution time, and B is a shape parameter. The reference dissolution profile was set with Dmax=85% of label claim, MDT=25 min, and B=1. A series of dissolution profiles was simulated by varying Dmax, MDT, and B values around those of the reference values as shown in Table 1.

In Vitro Dissolution Profile Comparisons

Comparisons were performed between each of the generated and the reference profiles by calculating the f2 similarity factor based on Eq. 1. Seven fixed time points between 0 and 60 min (5, 10, 15, 20, 30, 45, and 60 min)

Table I The Scenarios of Dissolution Profile Generation

	Dmax (%)	MDT (min)	B	Justification
Reference	85	25	1	Weibull parameters for a common dissolution profile
Test	70,75,80,85,90,95,100	10,15,20,25,30,35,40	0.4,0.6,0.8,1,1.2,1.4,1.6	Around the reference values with intervals of 5 for Dmax and MDT and 0.2 for B

were taken from both simulated and reference profiles for comparisons. If two or more values were greater than 85%, only one in either reference or test was used for calculation. At minimum, four time points were used in the f2 calculation.

IVIVC Model and Convolution

The IVIVC shown in Eq. 3 was assumed to have a linear (time invariant) component, with an intercept of a1 and a slope of a2, and a nonlinear (time variant) component describing time(t)-shifting (b1) and time-scaling (b2). A_{vivo} and A_{vitro} are the amount *in vivo* absorbed and *in vitro* dissolved, respectively. In this study, the parameters were assumed to have the values as shown in Table 2. These values were taken from a successful IVIVC model presented in a New Drug Application (NDA).

$$A_{vivo}(t) = a1 + a2 \times A_{vitro}(b1 + b2 \times t) \tag{3}$$

The dissolution profiles, after scaling and time shift and scaling were convolved with a unit impulse response (UIR) function, which was selected as a one-compartment IV bolus model as shown in Eq. 4

$$C_p = \frac{Dose}{V} e^{-\frac{CL}{V}t} \tag{4}$$

where C_p is the plasma concentration at time t after administration of the Dose. V is the volume of distribution, and CL is clearance. In this study, V=18 L and CL= 1.25 L/h.

The plasma concentration profiles were obtained by convolution as shown in Eq. 5

$$r(t) = \int_0^t i(u)w(t-u)du \tag{5}$$

where the response r(t) is the convolution integral between the input i(t) and the unit impulse response w(t).

A numerical convolution algorithm for computing was adopted from the literature (13). Briefly, discrete pulses of the input i(t), in combination with points or segments of a unit impulse response function w(t), added up to points or segments of the response r(t). The generation of additional nodes and the smoothing were achieved by representing the time profile using the Weibull distribution model. Methods with unequal time steps were used to avoid excessive interpolation. The response was computed as a series of points r_k of the response function r(t), at prescribed time points. Point-area method (14) was used, which interpreted the values of i(t) and w(t) as trapezoidal areas. According to the convolution integral of Eq. 5, the generalized numerical algorithm can be written as shown in Eq. 6.

$$\begin{aligned} r_0 &= 0 \\ r_1 &= I|_0^1 \overline{w}|_0^1 \\ r_2 &= I|_0^1 \overline{w}|_1^2 + I|_1^2 \overline{w}|_0^1 \\ r_3 &= I|_0^1 \overline{w}|_2^3 + I|_1^2 \overline{w}|_1^2 + I|_2^3 \overline{w}|_0^1 \\ &\vdots \\ r_k &= I|_0^1 \overline{w}|_{k-1}^k + I|_1^2 \overline{w}|_{k-2}^{k-1} + \cdots + I|_{k-1}^k \overline{w}|_0^1 \end{aligned} \tag{6}$$

where the index k denotes discrete interpolation nodes representing either actual observations or values computed from a prescribed function (Weibull Eq. 2 for I and Eq. 4 for w). The values r_k were calculated by a triangular arrangement of terms, where each row computed a time point r_k as the sum of all inputs prior to k, multiplied by a corresponding interval of w(t); the interval between two consecutive nodes was denoted by lower and upper indices. Hence, each term in Eq. 6 was defined as a product of two corresponding intervals of i(t) and w(t), in reversed sequence and supplied at consistent time points. According to the point-area approach, relevant terms were defined as in Eqs. 7 and 8.

$$\overline{w}|_{k-1}^k = (w_{k-1} + w_k)/2. \tag{7}$$

$$I|_{t_{1-1}}^{t_1} = I(t_1) - I(t_{1-1}). \tag{8}$$

Table 2 The Parameters of IVIVC model[a]

Parameters	a1	a2	b1	b2
Values	0	3	8	0.5

[a] Parameters chosen from an approved NDA where an IVIVC was established

Determining Cmax, Tmax, AUC, CmaxR, TmaxDif and AUCR

After the *in vivo* concentration time profiles were obtained from the reference and simulated dissolution profiles, the maximum plasma concentration (Cmax), time to reach

maximum plasma concentration (Tmax), and area under the plasma concentration curve (AUC) for each profile were calculated. Cmax and Tmax were identified using an R function max (Version 2.10.1, R foundation for statistical computing), and the AUC was calculated according to Eq. 9.

$$\mathrm{AUC_t} = \int_0^t \mathrm{Cp}\cdot \mathrm{dt} \approx \Delta \mathrm{t}\cdot\left(\mathrm{Cp_{t0}}/2 + \mathrm{Cp_{t1}} + \mathrm{Cp_{t2}} + \cdots + \mathrm{Cp_t}/2\right) \tag{9}$$

where $\mathrm{Cp_{t0}}$, $\mathrm{Cp_{t1}}$, …, $\mathrm{Cp_t}$ refer to the concentrations at initial time point (0), time point 1 and so on, until time point t.

The AUC, Cmax, and Tmax were compared between those obtained from the simulated profiles and those from the reference profile. The Cmax ratio (CmaxR), AUC ratio (AUCR), and Tmax difference (TmaxDif) were calculated by using Eqs. 10, 11 and 12, respectively.

$$\mathrm{CmaxR} = \mathrm{Cmax_{sim}}/\mathrm{Cmax_{ref}} \tag{10}$$

$$\mathrm{AUCR} = \mathrm{AUC_{sim}}/\mathrm{AUC_{ref}} \tag{11}$$

$$\mathrm{TmaxDif} = \mathrm{Tmax_{sim}} - \mathrm{Tmax_{ref}} \tag{12}$$

where $\mathrm{Cmax_{sim}}$ and $\mathrm{Cmax_{ref}}$ are the Cmax of *in vivo* concentration-time profiles obtained from simulated and reference profiles, respectively; $\mathrm{AUC_{sim}}$ and $\mathrm{AUC_{ref}}$ are the AUC of *in vivo* concentration-time profiles obtained from simulated and reference profiles, respectively; and $\mathrm{Tmax_{sim}}$ and $\mathrm{Tmax_{ref}}$ are the Tmax of *in vivo* concentration-time profiles obtained from simulated and reference profiles, respectively.

Data Analyses

Dissolution profile similarities were judged by the value of the f2 similarity factor (whether it is less than 50). Bioequivalence was determined by CmaxR and AUCR. When both values were within the range of 0.8–1.25, the test and reference profiles were considered bioequivalent. The percentage of the simulated and reference dissolution profiles that were found similar at each Weibull parameter value and the percentage of the simulated and reference *in vivo* concentration-time profiles that were found bioequivalent at each Weibull parameter value were also calculated.

Since the dissolution parameters used for simulation determined the dissolution profile and the subsequent *in vivo* concentration-time profile, they further affected the *in vitro* similarities and *in vivo* bioequivalence. The effects of Dmax, MTD and B on f2, as well as AUCR and CmaxR, were examined using graphical techniques with the emphasis on the critical values for f2 (50), AUCR and CmaxR (0.8 and 1.25).

Computations

The R program (Version 2.10.1) was used for simulation of dissolution profiles, plots for each simulated profile and reference profile for a visual comparison, f2 computations, numerical convolution, plotting of the *in vivo* concentration-time profiles, calculations of the AUC, Cmax and their ratios (CmaxR and AUCR), and determinations of the *in vitro* similarities and *in vivo* bioequivalence. An R script was written to perform these tasks sequentially in a batch process manner. Specifically, the script used several layers of loops to handle the various combinations of dissolution profile parameters.

Once a dissolution profile was generated for a specific combination of parameters, the percent dissolved values along with the corresponding time values were recorded in a data sheet. Simultaneously, a plot of percent dissolved against time for this specific profile and the reference was produced. At the plot region or the margin, the parameters used for generating this profile (Dmax, MDT, and B) and the calculated f2 value for the comparison between this and the reference profiles were noted. Subsequently, this dissolution profile was scaled and shifted according to the IVIVC model (Eq. 3) to obtain its *in vivo* dissolution profile, which then was convolved with the UIR function (Eq. 4) using the algorithm shown in Eqs. (5) and (6). From the obtained *in vivo* concentration-time profile, AUC, Cmax, Tmax, AUCR, CmaxR and TmaxDif were calculated. At the same time, a plot of concentration *vs.* time for the simulated and reference profile was generated, and the dissolution parameters used to evolve this profile as well as the calculated AUC, Cmax, Tmax, AUCR, CmaxR and TmaxDif, were noted.

RESULTS

The Impact of MDT, B and Dmax on f2 Similarity

A total of 343 dissolution profiles were simulated with different combinations of the Weibull parameters MDT, B and Dmax, as described in the Methods section. The simulated profiles were accommodated in seven figures—one for each level of Dmax. Each figure contains 49 panels. Each row represents an MDT level, and each column represents a B level. Figure 1 illustrates the dissolution profiles for Dmax=85. The figures of the other dissolution profiles are located in the Supplementary Material (Figures S1–6). In panel 25 of Fig. 1, the test and reference profiles have the same Weibull parameters; thus, an f2

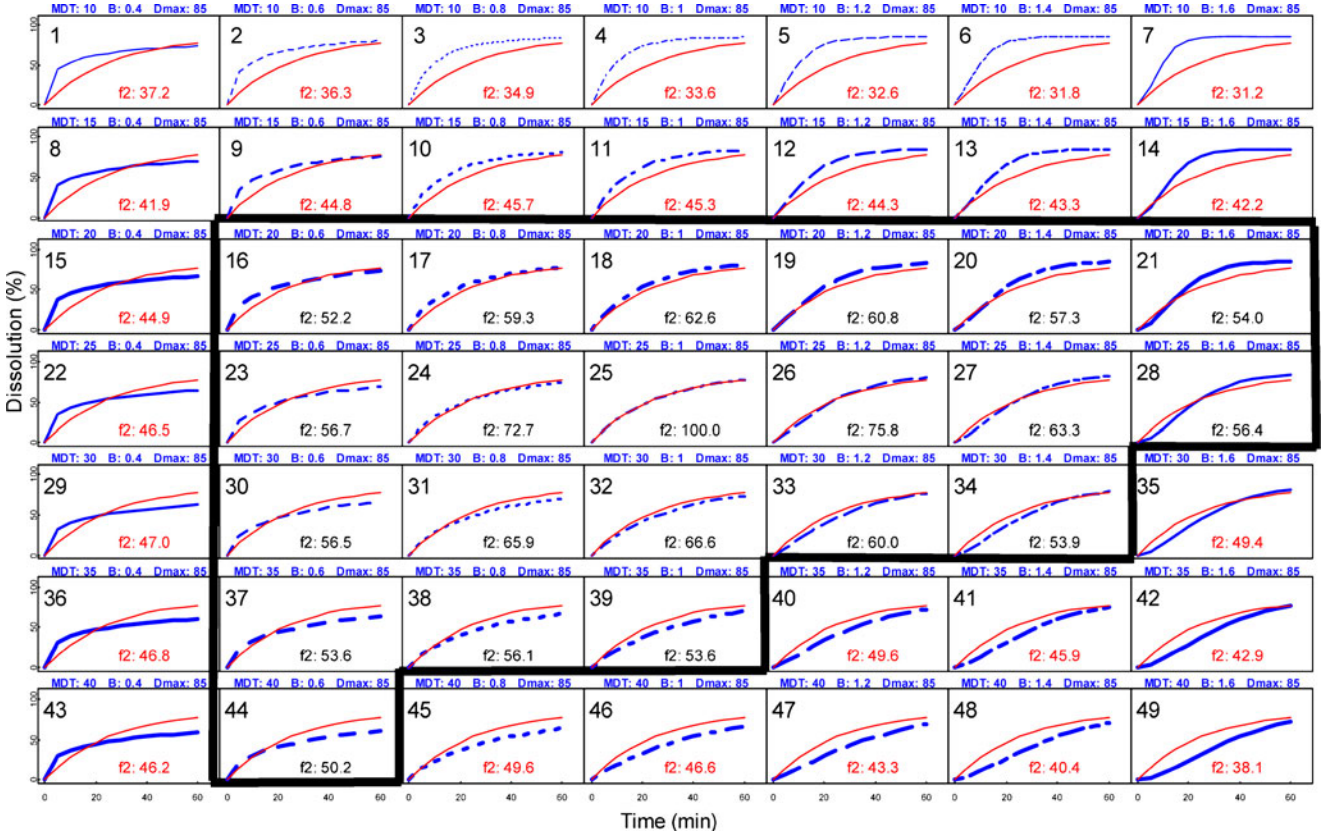

Fig. 1 Dissolution profile comparisons for Dmax = 85. Blue line: the simulated dissolution profile. Red solid line: reference dissolution profile. The parameters used for simulation are labeled at the top of each panel. The f2 similarity value is noted in each panel. f2 values ≥50 are colored black, while f2 values < 50 are colored red. The solid black border around the panels outlines the profiles which are similar.

value of 100 was obtained, which indicates an exact match between the simulated and the reference profiles as expected. For a given Dmax value, when either MDT or B parameters deviated from those of the reference, the f2 value was less than 100. It can be observed from Fig. 1 as well as in Figures S1–6 that when MDT deviated by more than 10 (MDT < 15 and MDT > 35), the f2 values were less than 50. Additionally, when B deviated by 0.6 (B = 0.4 or B = 1.6), the f2 values were less than 50. Overall, 132 (38.5%) of the 343 simulated dissolution profile comparisons demonstrated f2 similarity.

Table 3 lists the percentages of the simulated and reference dissolution profiles that were found similar at each Weibull parameter value of the simulated profiles. The table demonstrates that the simulated and reference profiles were most similar (75% of the time) when the simulated profiles had MDT = 25. Even when the simulated profiles had MDT = 20 or MDT = 30, 53.1% of the profile comparisons demonstrated similarity between reference and simulated profiles. When the simulated profiles had B = 1, 55.1% of the simulated profiles were found similar to the reference profiles. Also, when B = 0.8 or B = 1.2, 53.1% and 49.0%, respectively, of the profiles had f2 similarity. Furthermore, when the simulated profiles had Dmax = 85, only 42.9% of the simulated profiles were found similar to the reference profiles. Interestingly, when the simulated profile had MDT = 10 or B = 0.4, none of the simulated profiles were found similar to the reference profiles. These data show that it was more important for the dissolution profiles to have similar MDT values than similar B or Dmax values for the profiles to have f2 similarity.

Table 3 Percent of the Simulated and Reference Dissolution Profiles Found Similar at Each Weibull Parameter Value of the Simulated Profiles[a]

Dmax (%)	Percent Similar	MDT (min)	Percent Similar	B	Percent Similar
70	28.6	10	0.0	0.4	0.0
75	36.7	15	30.6	0.6	46.9
80	40.8	20	53.1	0.8	53.1
85	42.9	25	75.5	1	55.1
90	44.9	30	53.1	1.2	49.0
95	38.8	35	34.7	1.4	40.8
100	36.7	40	22.4	1.6	24.5

[a] Percent similar = Number of similar profiles / Total number of profiles. This value was calculated for each Dmax, MDT, and B for the simulated dissolution profile. The total number of profiles was 49 for each case.

The relationship between the f2 values and the Weibull parameters is displayed in a series of contour plots in Fig. 2. When MDT and B took the values enclosed by the thick solid lines labeled "50," the f2 value for the comparison between the simulated and the reference profile were greater than or equal to 50. Any combination of the parameters that fell in the region enclosed by the thick solid line resulted in a dissolution profile that is considered similar to the reference. We defined this region as the *f2 Similar Region*. The combination of the parameters outside of this region resulted in the simulated and reference dissolution profiles being dissimilar.

The Effect of Weibull Parameters on AUCR, CmaxR, TmaxDiff

The simulated dissolution profiles were scaled and shifted according to the IVIVC model and convolved with the UIR function as outlined in the Methods section. A total of 343 *in vivo* concentration-time profiles were generated similarly to the *in vitro* dissolution profiles. Figure 3 illustrates the *in vivo* concentration-time profiles for Dmax = 85; each panel corresponds to a panel in Fig. 1. The figures of the other *in vivo* concentration-time profiles are located in the Supplementary Material (Figs. S7–12). Panel 25 of Fig. 3, which corresponds to panel 25 in Fig. 1, where f2 = 100, demonstrates an overlap between the simulated and the reference profile. As expected, AUCR = 1, CmaxR = 1, and TmaxDif = 0 were calculated for this profile comparison. The general trend that we observed in the simulated/reference profile comparisons was that as MDT increased, AUCR and TmaxDiff increased; however, CmaxR decreased. Also as B increased, AUCR, CmaxR and TmaxDiff increased.

The Impact of Weibull Parameters on *In Vivo* Bioequivalence

In Fig. 3 and Figs. S7–12, the AUCR and CmaxR values shown in each panel indicate whether the simulated profiles were bioequivalent to the reference profile. It is important to point out that the failure of bioequivalence might be due to CmaxR, AUCR, or both. To distinguish amongst the cases, the AUCR and CmaxR values within the bioequivalence range (0.8–1.25, inclusive) were colored black, while the values out of range (<0.8 or >1.25) were colored red in these figures.

The relationship between the Weibull parameters and AUCR and CmaxR is displayed in Figs. 4 and 5, respectively. The contour lines represent AUCR and CmaxR values of 0.80 and 1.25, respectively. As shown in Fig. 4, certain combinations of MDT, B and Dmax caused the AUCR to fall outside of the boundary of 0.80 and 1.25 (those AUCR values in the regions around the dashed lines). For three out of the seven panels, the contour line for 1.25 was not visible. It is interesting to note that the contour lines for CmaxR (Fig. 5) were diagonal to those for AUCR (Fig. 4). The four contour lines (two for AUCR and two for CmaxR) enclosed a region, within which both AUCR and CmaxR fell within the 0.80–1.25 limits. Any combinations of the Dmax, MDT and B in this area resulted in an *in vivo*

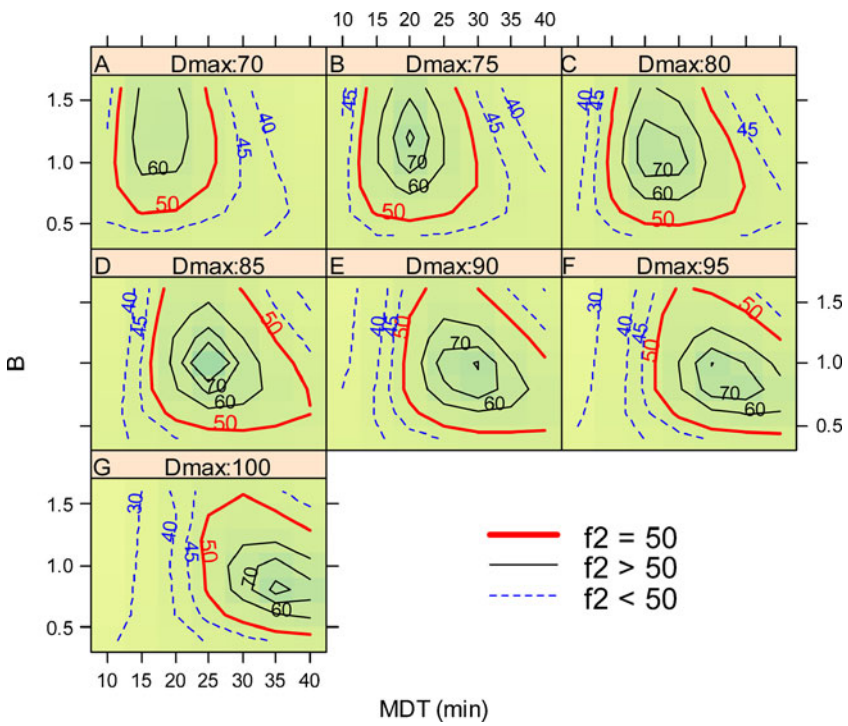

Fig. 2 Effect of MDT, B and Dmax on f2 similarity values. The thick solid line represents the f2 value of 50 as labeled. The thinner solid lines stand for the f2 values >50, while the dashed lines indicate the f2 values <50.

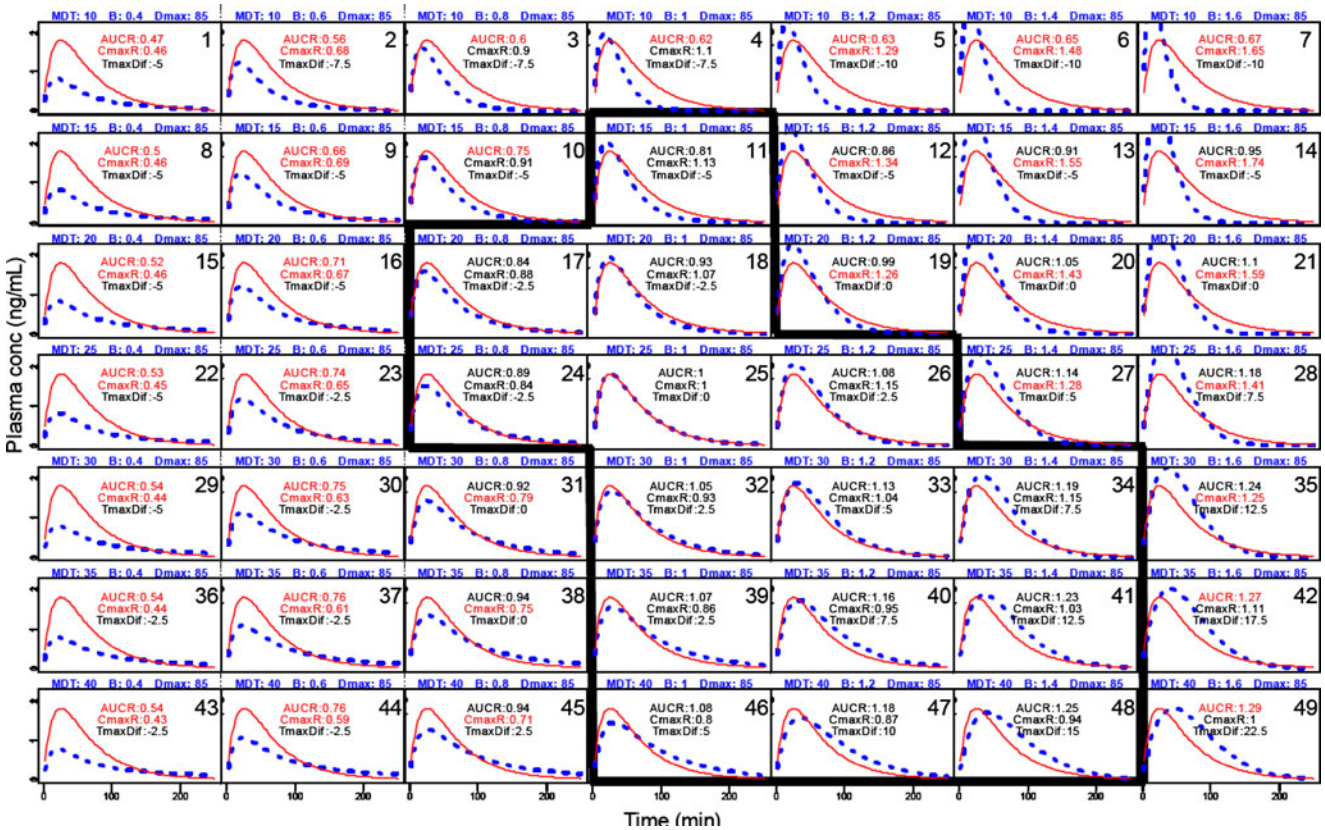

Fig. 3 Comparisons of *in vivo* concentration-time profiles for Dmax = 85. At the top of each panel are the model parameters used for simulating the *in vitro* dissolution from which the *in vivo* profile were obtained. Red solid line: the reference profile. Dotted line: simulated profile. The AUCR, CmaxR and TmaxDif are noted in each panel. The solid black border around the panels outlines the profiles which are bioequivalent.

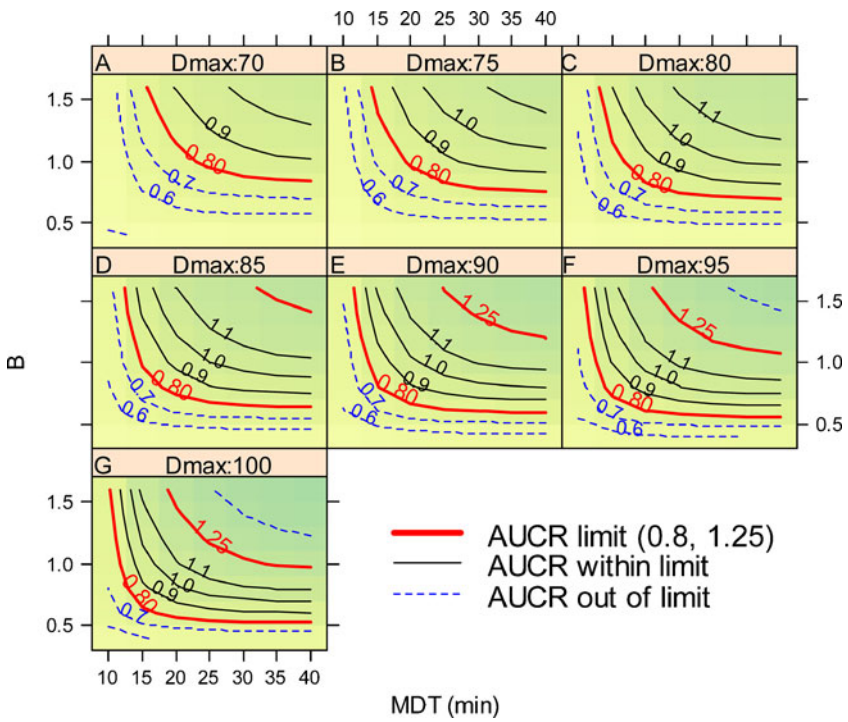

Fig. 4 Effect of MDT, B and Dmax on the AUC ratios. When the MDT and B take the values between the two thick lines, the AUC ratio is in the 80–125% range of bioequivalence. The combination of the parameters outside of the range enclosed by the two thick lines would result in the simulated profile being not bioequivalent to the reference profile as far as the AUC is concerned.

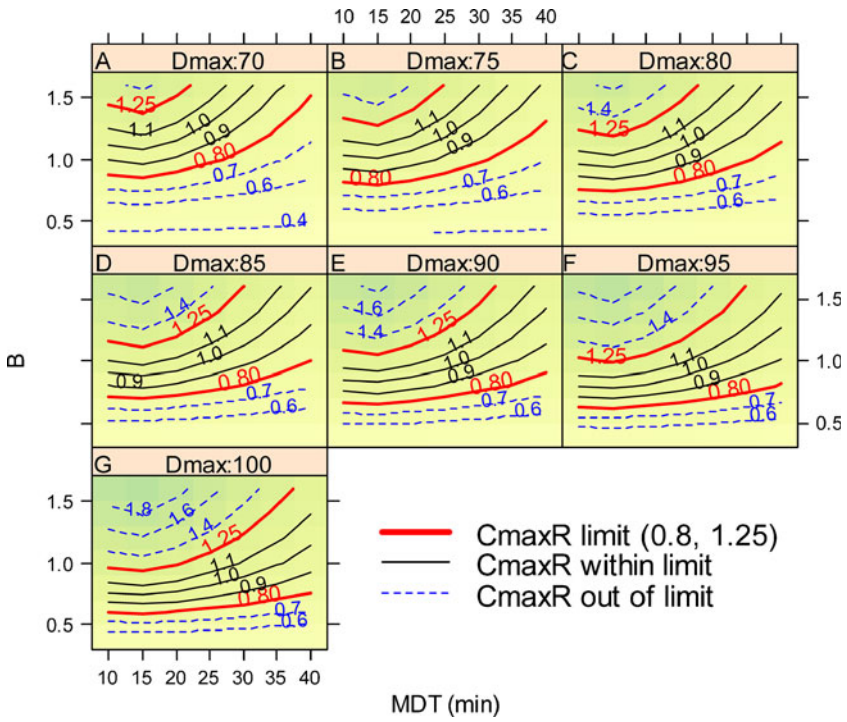

Fig. 5 Effect of MDT, B and Dmax on the Cmax ratios. When the MDT and B take the values between the two thick lines, the Cmax ratio is in the 80–125% range of bioequivalence. The combination of the parameters outside of the range enclosed by the two thick lines would result in the simulated profile being not bioequivalent to the reference profile as far as the Cmax is concerned.

profile which was bioequivalent to the *in vivo* profile obtained from the reference dissolution profile. We defined this region as the *Bioequivalence Region*. This region is presented in Fig. 6. As one can observe from Figs. 4, 5 and 6, the contour lines shift in each panel. Therefore, the Weibull parameters had an impact on *in vivo* bioequivalence. Overall, 88 (25.6%) of the 343 simulated *in vivo* profiles were bioequivalent to the reference profile.

Table 4 lists the percentages of the simulated and reference *in vivo* concentration-time profiles that were found bioequivalent at each Weibull parameter value of the simulated profiles. The table demonstrates that the simulated and reference *in vivo* profiles were most bioequivalent (55.1% of the time) when the simulated profile had B = 1. When the value of B was less than 0.6, 0% of the *in vivo* reference and simulated profiles were bioequivalent. There

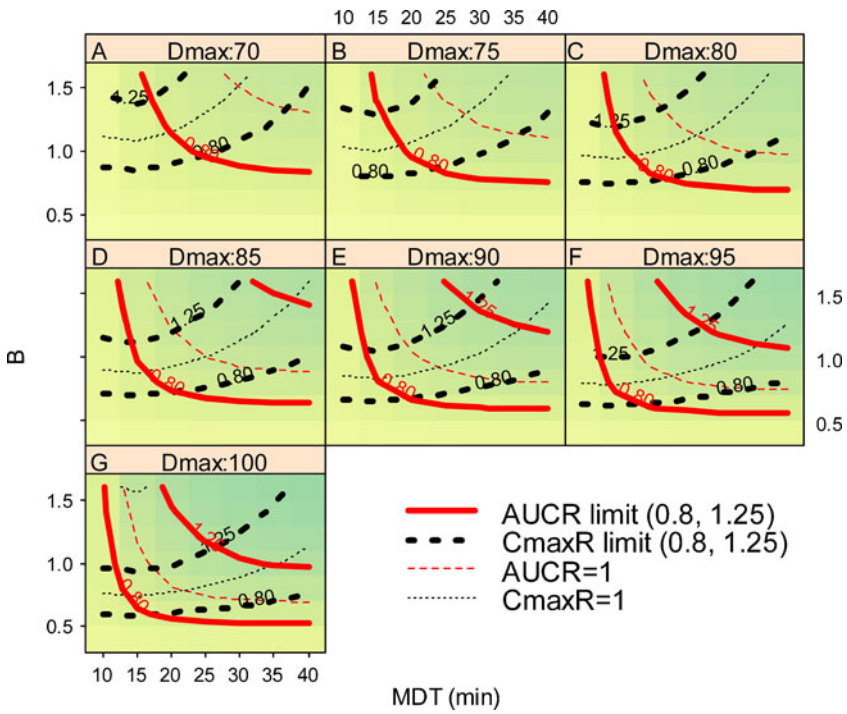

Fig. 6 Effect of MDT, B and Dmax on AUC ratios and Cmax ratios. When the MDT and B take the values enclosed by the AUCR limit lines (thick solid lines) and the CmaxR limit lines (thick dashed lines), the simulated *in vivo* concentration-time profiles are bioequivalent to the reference profile. The combination of the parameters outside of the region enclosed by the four lines would result in a profile which is not bioequivalent to the reference.

Table 4 Percent of the Simulated and Reference *In Vivo* Concentration-Time Profiles Found Bioequivalent at Each Weibull Parameter Value of the Simulated Profiles[a]

Dmax (%)	Percent Bioequivalent	MDT (min)	Percent Bioequivalent	B	Percent Bioequivalent
70	24.5	10	0.0	0.4	0.0
75	30.6	15	8.2	0.6	0.0
80	32.7	20	26.5	0.8	32.7
85	30.6	25	34.7	1	55.1
90	24.5	30	42.9	1.2	38.8
95	20.4	35	34.7	1.4	30.6
100	16.3	40	24.5	1.6	22.4

[a] Percent bioequivalent = Number of bioequivalent profiles / Total number of profiles. This value was calculated for each Dmax, MDT, and B for the simulated *in vivo* concentration-time profiles. The total number of profiles was 49 for each case.

were no Dmax or MDT values that produced bioequivalent profiles greater than 45% of the time. Thus, the data demonstrate that it was more important for the reference and simulated profiles to have the same B values than the same MDT or Dmax values for the profiles to be bioequivalent.

The Impact of Weibull Parameters on the Consistency Between f2 Similarity and *In Vivo* Bioequivalence

Comparing the values in Tables 3 and 4, it is apparent that there is not complete agreement between f2 similarity and *in vivo* bioequivalence, since the percent similar dissolution profiles do not exactly match the percent bioequivalent *in vivo* profiles. For example, when the simulated profiles had MDT = 25, 75.5% of the dissolution profiles had f2 similarity, while only 34.7% of the *in vivo* profiles were found bioequivalent. In fact, 80 (60.6%) of the 132 f2 similar profiles did not show *in vivo* bioequivalence. Also, 38 (40.9%) of the 88 bioequivalent profiles did not have f2 similar dissolution profiles. Therefore, we plotted the f2, AUCR, and CmaxR data to further characterize the consistency between f2 similarity and *in vivo* bioequivalence.

Overlapping plots for f2, AUCR and CmaxR are shown in Fig. 7. At Dmax = 85 (the same as reference value), most of the *Bioequivalence Region* overlapped with the *f2 Similarity Region*. However, there were instances when these two regions did not match as shown in panel D of Fig. 7. In one case, B played a major role. For example, when B deviated from the reference value to a certain extent (B < 0.75 or B > 1.5), there was a possibility that although the simulated dissolution profile was similar to the reference based on the f2 comparison, the corresponding *in vivo* profile was not bioequivalent to the reference due to Cmax failure. Graphically, as presented in panel D in Fig. 7, this case included the region enclosed by the dotted thick lines labeled "0.80" (Cmax lower bound contour line), the lower part of dashed thick line labeled "50" (f2 = 50 contour line), the region enclosed by the dotted thick line labeled "1.25" (Cmax upper bound contour line), and the upper part of dashed thick line labeled "50" (f2 = 50 contour line).

In another case, MDT played a major role in conjunction with B. Two scenarios were observed: 1) *in vivo* bioequivalence without f2 similarity and 2) f2 similarity without *in vivo* bioequivalence. In the first scenario, when MDT > 40 min or MDT < 17 min, the combinations of MDT and B resulted in a dissolution profile that was dissimilar to the reference, although the corresponding *in vivo* profile was bioequivalent to the reference. In contrast to Scenario 1, when 17 < MDT < 22 min, there was a small triangle region in which the combinations of MDT and B resulted in an *in vivo* profile that was not bioequivalent to the reference, while it was judged to be similar by f2 comparison.

When Dmax deviated from the reference value (Dmax = 85), the relative positions of the *Bioequivalence Region* and the *f2 Similarity Region* shifted. When Dmax increased, the *f2 Similarity Region* (indicated by the thick dashed lines) shifted to the lower right. However, the *Bioequivalence Region* (enclosed by the thick solid and dotted lines) behaved differently. While the two boundary lines of CmaxR shifted in the same direction as the *f2 Similarity Region*, the boundary lines of AUCR shifted to the lower left. In addition, it not only shifted, but also narrowed. Thus, all three Weibull parameters impacted the consistency between f2 similarity and *in vivo* bioequivalence.

DISCUSSION

This study investigated the consistency between *in vitro* dissolution profile comparison using the f2 equation and *in vivo* bioequivalence evaluation for extended release formulations with different combinations of dissolution profile parameters.

We used the Weibull model to model the dissolution profiles because it is a widely used model that accurately describes drug release phenomena (11). In this study, we determined that all three Weibull parameters impacted the consistency between f2 similarity and *in vivo* bioequivalence. As demonstrated from Table 4, under the setting of this study, it was more important for the simulated and reference profiles to have the same B values than the same MDT or Dmax values for the *in vivo* profiles to be bioequivalent. When the simulated profiles had the same value of B as the reference profile (B = 1), the corresponding *in vivo* profiles were bioequivalent 55.1% of the time. A

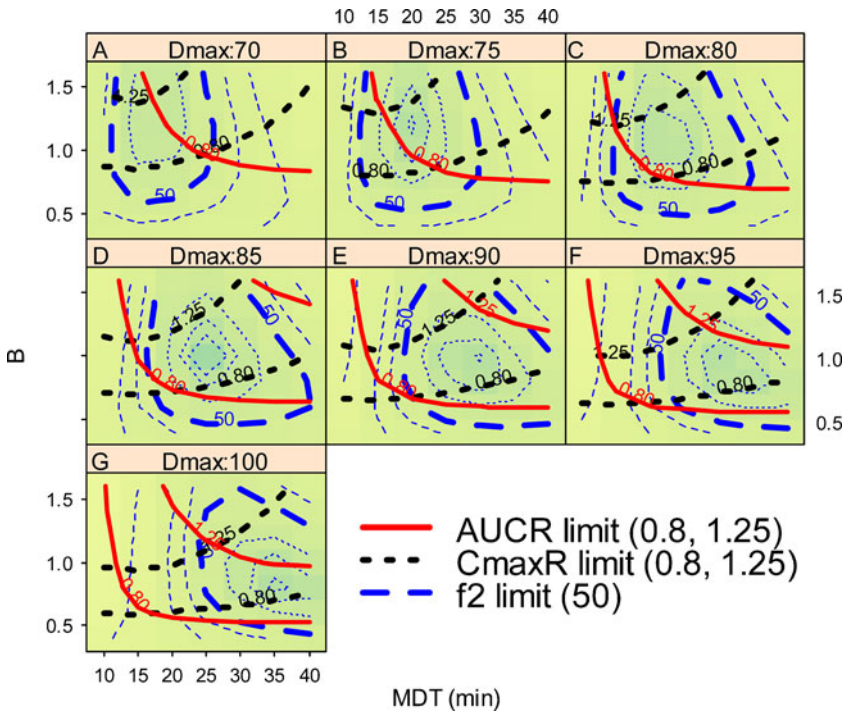

Fig. 7 Effect of MDT, B and Dmax on the AUC ratios, Cmax ratios, and f2 comparisons. When the MDT and B take the values enclosed by the two thick red lines (for AUCR) and the two black thick lines labeled (for CmaxR), the simulated *in vivo* concentration-time profiles are bioequivalent to the reference (bioequivalence region). The blue thick lines enclose a region in which the combinations of MDT, B and Dmax produce dissolution profiles similar to the reference profile (f2 similarity region).

20% deviation in the B value significantly decreased the amount of profiles that were bioequivalent to 30–40%.

In the Weibull model, B is the shape parameter. The deviation in the value of B compared to the reference value represents the difference of the dissolution profile shapes. As the B values increase from 0.4 to 1.6, the shape of the dissolution curves change (Fig. 1 and Figs. S1–6). At the lower values of B, the simulated profile was above the reference profile at the early time points and below the reference profile at the later time points. At the higher values of B, the simulated profile was below the reference profile at the early time points and above the reference profile at the later time points. These differences reflect the changes of the sigmoidicity of the profile. In general, $B=1$ represents a monoexponential as a special case, $B>1$ describes a "sigmoid" profile retarded at the beginning, and $B<1$ represents a profile faster at the beginning but retarded at the tail (15). In this study, the reference profile had a B value of 1; therefore, the reference profile followed an exponential distribution. When the B value of the simulated profile deviated from the reference value, the ratio between the rates at the beginning and at the tail changed. The rate at different stages either accelerated or decelerated with time. The acceleration or deceleration of the dissolving rate *in vitro* could translate to *in vivo* dissolution/absorption rate changes and directly affect Tmax and Cmax of the *in vivo* profile as shown in Fig. 3.

When Dmax deviated from the reference value, the *Bioequivalence Region* and the *f2 Similarity Region* shifted in different directions as shown in Fig. 7. As a consequence of these shifts, the overlap between the *Bioequivalence Region* and the *f2 Similarity Region* narrowed and the inconsistency became more significant. In Fig. 7, panels A and G, which had the most extreme deviations in the Dmax value, showed the least area of overlap in the *Bioequivalence Region* and the *f2 Similarity Region as* compared to other panels in this figure. In these two panels, most of the combinations of Dmax, MDT and B in the *f2 Similarity Region* resulted in non bioequivalence to the reference while the dissolution profiles obtained from most of the *Bioequivalence Region* was judged not to be similar to the reference by f2 comparisons.

The influence of Dmax is understandable because it is a parameter that reflects the plateau of the dissolution profile (i.e., the maximum percent of the drug dissolved). The difference in Dmax between the reference and simulated profiles may indicate the difference of completeness of the dissolution/absorption of the drug. It is plausible that if two formulations have significantly different completeness of absorption, they would likely not be bioequivalent, since similarity in the extent of absorption is the key component for bioequivalence. Our observations imply that the plateau of the dissolution curve is an important parameter because it indicates the completeness of drug dissolution/absorption. Based on our results, a 10% deviation in Dmax was roughly the cutoff point to assure the *in vivo* bioequivalence between two formulations using the f2 dissolution profile comparison approach.

The MDT also played a role in the bioequivalence of the profiles. Undoubtedly, varying dissolution times can affect

the Cmax and Tmax of *in vivo* profiles and impact on the bioequivalence of the reference and simulated profiles.

Overall, we found that most of the inconsistency between f2 similarity and *in vivo* bioequivalence occurred when the simulated and reference dissolution profiles crossed or when the simulated dissolution profile was above or below the reference dissolution profile. The data showed two different outcomes when the simulated dissolution profile was above or below the reference dissolution profile. In one case, the dissolution profiles in Fig. 1 panel 21 had f2 similarity; however, the corresponding *in vivo* profiles (Fig. 3 panel 21) were not bioequivalent. This non-bioequivalence was due to the Cmax of the simulated profile being significantly greater than the reference profile. Interestingly, the dissolution profiles in Fig. 1 panel 11 did not show f2 similarity when the simulated dissolution profile was above the reference profile. However, the corresponding *in vivo* profiles (Fig. 3 panel 11) were bioequivalent because the simulated profile Cmax was similar to the reference Cmax. An example of when the simulated and reference dissolution profiles cross is illustrated in Fig. 1 panel 28. In this case, the simulated and reference dissolution profiles had f2 similarity; however, the corresponding *in vivo* profiles (Fig. 3 panel 28) were not bioequivalent due to Cmax failure. These examples demonstrate the importance of the shape and plateau of the dissolution curves in the consistency between f2 similarity and *in vivo* bioequivalence.

The number of false positive cases (i.e. the test and reference dissolution profiles pass the f2 test, whereas the test and reference *in vivo* profiles are determined to be not bioequivalent) is 80 among a total of 343 cases, which is 23.3%. The number of false negative cases (i.e. the test and reference dissolution profiles fail the f2 test, but the test and reference *in vivo* profiles are determined to be bioequivalent) is 38 out of 343 cases, which is 11.1%. A 23.3% false positive rate seems to be high. However, this does not necessarily mean that the f2 prediction on bioequivalence has a high false positive rate. As a simulation study, a variety of scenarios were simulated, and some of them may be uncommon, such as the cases in which the test and reference profiles had significant shape differences. One of the major intentions of this study was to identify and characterize these cases. In case they appear, the cases can be quickly recognized, and, thus, appropriate action can be taken to avoid mistakes.

The mechanistic interpretations discussed herein underscore the importance of the rate of absorption and the completeness manifested by the dissolution behavior. These parameters are not considered in the f2 calculation. The f2 equation only considers the distances between the two dissolution curves. The inconsistency between the results from the f2 similarity and the bioequivalence comparison is most likely due to this fact. These interpretations suggest that if a test formulation is truly similar to the reference product, they will be bioequivalent in an *in vivo* study if the difference between the dissolution profiles stems from a random variation rather than a significant plateau difference or an obvious shape difference. Therefore, to ensure real similarity between the test and reference products, when the f2 equation is used, the plateau of the test product should not differ more than 10% from the reference. Additionally, the general shape of the dissolution profile of the test product should not be significantly different from the reference. The completeness of the dissolution profile, which is the major component of the extent of dissolution/absorption, and the shape of the curve, which can be translated into the rate of *in vivo* dissolution/absorption, are important factors because the major concerns of bioequivalence are the differences in rate and extent of the drug available at the site of action.

The observations from this study were from a special drug with a set of special parameters. Also, only one IVIVC model was used. Therefore, the interpretation may not be extrapolated to all cases. Note that the IVIVC model was assumed in this study. In reality, this relationship may not exist. Nevertheless, in order for an oral drug in a solid dosage form to be absorbed, the dissolution is the first and important step. Using a reasonable model to approximate this step seems appropriate, although it is not necessary to use the same models as in this study. It is important to note that we assumed that dissolution was the rate-limiting step in this study. Other situations including cases for immediate release formulations, cases when absorption is the rate-limiting step, and cases where dissolution and absorption are at the similar rate should also be considered. It is also important to point out that multiple factors/variables can have significant impact on the outcomes and conclusions of the simulation and require further investigation. Among them are (1) the selected IVIVC model (such as linear or non-linear relationship), (2) *in vitro* dissolution model (zero-order, first-order, Higuchi, exponent, bimodal, etc.), (3) *in vitro-in vivo* relationship (such as lack of an IVIVC, rank order, or dissolution under-discriminating or over-discriminating), and (4) UIR model (such as compartment, non-compartment, spline, or polynomial). Further investigations using different UIR models, dissolution models and IVIVC models are underway. In the meantime, we generalize the major conclusions obtained from this study, which are independent of the models (parameters) and should be considered for general cases.

One contribution of this study is that we quantitatively demonstrated the importance of the extent and the rate of absorption using modeling techniques. Furthermore, our proposed 10% criterion in difference in the plateau levels is consistent with the f2 similarity criterion, because the cutoff value of 50 for f2 is set based on an average difference of

10% at all measured time points. Although it is difficult to set a numerical value to evaluate the shape difference, in general, if a crossover between the test and reference curves is observed, caution should be exercised. Another contribution of this study is a method to connect the *in vitro* results to *in vivo* performance. We created an R script for the purposes of investigating general biopharmaceutics questions. When it was written, we had a biopharmaceutics evaluation system in mind such that it could handle dissolution profiles with diverse parameter values as well as various IVIVC models. With the freedom to get a variety of desired *in vitro* dissolution and *in vivo* concentration profiles, the system can serve as a bridge to link the product manufacturing variables to clinical performance.

In this regard, the system may be useful for drug development, especially for post-approval manufacturing changes. An important aspect for efficient drug development is to limit unnecessary human testing. The application of the system presented here could lead to more efficient bioequivalence testing. If an IVIVC has already been established for the reference drug, the system can be used directly by plugging in dissolution profile and IVIVC model parameters into the system to select a set of optimal parameters as targets. In many cases, an IVIVC has not been established, but an *in vitro-in vivo* relationship (IVIVR) is shown. Under these situations, using the system to predict the worst-case scenario in order to avoid pitfalls would be possible and helpful. Even without available IVIVC-related information, one can explore such a relationship with the help of biorelevant dissolution (16–20). Various scenarios could be explored in order to determine the optimal parameters to achieve a test product that is bioequivalent to the reference product.

CONCLUSIONS

In this study, we have demonstrated the general consistency between *in vitro* dissolution comparison using the f2 factor and *in vivo* bioequivalence. The results indicate that dissolution profiles that are judged similar using the f2 factor may not always be bioequivalent when tested *in vivo*. On the other hand, *in vitro* dissolution profiles judged dissimilar by the f2 factor may sometimes generate *in vivo* bioequivalent profiles.

This study emphasizes the importance of evaluating the shape and the completeness of *in vitro* dissolution curves when f2 is used to determine the similarity between different formulations, since the completeness of dissolution relates to the extent of drug absorption *in vivo*, and the shape of a dissolution curve is translated to the rate of drug absorption *in vivo*. In particular, when there is a difference of more than 10% in the plateau levels between dissolution profiles of the test and reference product or when the two dissolution profiles cross, there is a greater likelihood for the test product to be not bioequivalent to the reference product, although f2 similarity has been demonstrated. Under these circumstances, caution must be exercised in drawing conclusions.

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