Simulating/predicting outcome of a human bioavailability study from a dissolution test: A simple and practical approach

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A method based on convolution technique has been described earlier to predict plasma drug concentration time (C-t) profiles. This article describes further refinement of the method for a more realistic representation of a human bioavailability study outcome by including variabilities in stomach emptying time and bioavailability factor (F). The advantages of such refinement are discussed including setting physiologically relevant specifications for dissolution testing.

Drug dissolution tests are conducted to evaluate drug dissolution/release characteristics of pharmaceutical products (e.g. tablets and capsules) in humans or in vivo. As often it is not possible to directly measure in vivo drug dissolution, it is indirectly measured using plasma drug concentration-time (C-t) profiles, which are dependent on the in vivo dissolution. Therefore, in principle, in vitro dissolution which relates to in vivo dissolution, which in turn relates to C-t profiles forms the theoretical basis of linking the in vitro dissolution results to C-t profiles.

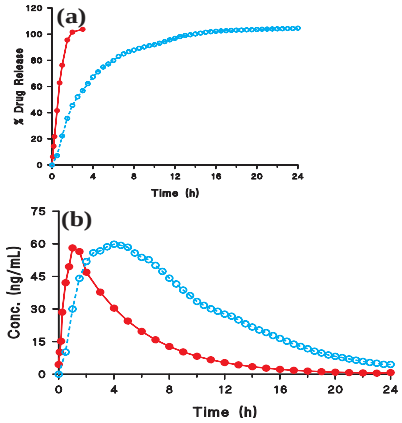

Figure 1: Dissolution profiles (a) of 60 mg IR tablet and 120 mg ER capsule products and corresponding calculated C-t profiles (b) using convolution technique.

Conversion of dissolution results or profiles into C-t profiles requires a mathematical procedure known as convolution. In this case, dissolution results are merged with the pharmacokinetic parameters of the drug under consideration which provides the C-t profiles. The theoretical background of the convolution methodology and its applications are described elsewhere (see links 1, 2). An example of such a conversion is shown in Figure 1 where dissolution results from two diltiazem (IR and ER) products are converted to C-t profiles. Figure 1 shows the conversion of the dissolution profiles, which could be from the dissolution results of a single dosage unit or average values from multiple dosage units. The prediction provides a very good estimate of C-t profiles of the products based on the estimates of C_{max} and AUC (i.e. Area Under the C-t profile or Curve).

A more accurate representation of the outcome of a human bioavailability/bioequivalence study based on a C-t profile, however, requires further adjustments. For clarity and differentiation, let us call the C-t profile from a human bioavailability study as a bio-profile and the one described above obtained from dissolution results, based on a single dissolution profile, as the base- (or basic) profile. For predicting the base-profile, the dissolution and absorption steps are assumed to occur immediately and concurrently, therefore, the base-profile will always show a relatively shorter T_{max}, compared to the one from the human bioavailability studies i.e. bio-profile where delays or lags are observed. Furthermore, the base-profile also assumes a constant (average) drug absorption (bioavailability factor or F) which is also not a realistic assumption. In addition, the base-profile does not reflect the inter-subject variability factors for both (lag time and bioavailability), which can also alter the overall C-t profiles significantly.

The objective of this article is to suggest an approach to include these factors such as lag time, F and inter-subject variability to simulate a more realistic representation of a C-t profile from a human bioavailability study.

Theoretical Considerations:

Simulating delay and variability in the T_{max}: To align with the delayed T_{max} observed in the human bioavailability studies, it is commonly suggested that one may use a lagging or scaling factor i.e. to add a fixed time interval to every sampling time to move the C-t profiles to the right on the time scale. However, there are at least two problems with this approach: (1) what that number (lag time) should be and how it should be determined. (2) If such an approach is followed then

obviously one will not observe any variability in the T_{max}, which would not be representative of a human study.

To address these questions, let us first consider what causes this lag time. To answer this question, one should consider the differences of in vitro dissolution and in vivo dissolution/absorption environments. For in vitro testing, the analyst has one vessel where the product is introduced and the drug is dissolved. However, in vivo, there are two vessels (compartments) i.e. stomach and small intestine. The product is introduced, or delivered, into the stomach where the product may, or may not, release the drug. The product/drug, however, must be transferred to the other vessel (small intestine) where it should be dissolved and then absorbed. Therefore, the transfer of the product from the stomach to the small intestine causes the lag time in human bioavailability studies which is not observed in vitro. In physiological terminology, the time required for the transfer of the stomach content, which includes drug/product as well, from the stomach to intestine is known as "stomach emptying time". The stomach emptying time comes with an inter-subject variability component. Therefore, one should expect to see lag times and variations in the appearance of drug in blood which are correspondingly reflected in the T_{max} values. As an example, a value of 80.5±22 minutes (±SD) has been reported (Link) for stomach emptying time in humans which may be used to reflect the lag time.

Simulating variability in C_{max}: The second set of variability in human bioavailability studies comes from variable drug absorption from the GI tract, which is reflected as variability in the absolute bioavailability (F) of the drug. This variation often reflects degradation of the drug in the GI tract and/or metabolism of the drug by the liver. To simulate this variability one would use the variability in F values for the ***drug*** which may be obtained from literature. For example, the reported variability for diltiazem is around 23% (CV) which may be introduced or combined with the dissolution results.

The next step is how should one introduce different stomach emptying times and bioavailabilities for an in vitro dissolution experiment, in a simple and practical way?

Perhaps the easiest approach to simulate the stomach emptying time is to stagger the times of introduction of a product into the dissolution vessels. For this, imagine that an analyst has to run a dissolution test using a 24-vessel apparatus, representing 24 human subjects, where one tablet is introduced in each vessel at different times. This staggering will represent the availability of drug product in the intestine with an average time of 80.5±22 minutes (±SD), as noted above. The actual staggering times can be obtained from Excel spreadsheet software using a randomization function with a mean and SD given above. The rest of this "virtual" dissolution experiment will be conducted as usual and dissolution results will be converted to C-t profiles ***individually*** for every vessel using the convolution technique, as described for the base-profile. Therefore, in the end, one will have 24 C-t profiles with different T_{max} values representing stomach emptying times of individuals. All these C-t profiles will have the same shape except for the lag times reflecting the staggering of the tablet for dissolution testing or stomach emptying times.

For simulating variations in bioavailability, one can imagine that the dissolution analyst is given 24 filters to use when withdrawing samples from the dissolution vessels. Each filter adsorbs, at random, a different amount of drug thus allowing partial amounts of the drug to go into filtered solutions representing different amounts of drug appearing in blood/plasma. For this particular example it is assumed that the filter will release (not adsorb) on average 44%±10(±SD) of the drug, representing average F (bioavailability) and variation in the F for diltiazem. Again, randomization of the "F" can be done using Excel spreadsheet software given the mean and SD values.

Practical Demonstration:

To demonstrate application of this approach, the drug dissolution data employed is the same which was used to develop the base-profiles, where drug dissolution characteristics of 60-mg IR tablets and 120-mg capsules were evaluated. The experimental conditions were common for both products and were: 900 mL of water as the medium, maintained at 37 °C, with crescent-shape spindle set at 25 rpm. For complete details of the experimental conditions along with the procedure used for conversion (or convolution) to C-t profiles, please see the link (1).

On the practical side, the analyst should first calculate a C-t profile (calculated drug concentrations vs times) from a dissolution study using a convolution technique which has been described in the publication (1). For this demonstration, the data, i.e. calculated concentration and times, used are the same which have been reported in the publication (1).

On a separate (work) sheet one should have one column for times followed by 24 columns, representing 24 subjects, containing 24 repeats of the previously calculated plasma concentrations but values in each column are to move down by respective staggered time. All initial cells should be filled with zero plasma drug concentrations.

This is followed by another 24 columns of calculated concentrations but adjusted with the bioavailability factor for the respective subjects. The method used for calculation of adjusted concentration is as follows: As the base-profile was calculated using an average bioavailability (F) of 44%. Therefore, if 44% bioavailability shows certain concentration in the staggered emptying time area ("X" value in a cell at a time corresponding to subject number) then a different bioavailability ("Y" in the staggered F area) will show concentration={(X*Y)/44}. Each cell in the 24 columns will utilize the formula to calculate the respective concentrations depending on the bioavailability of that column/subject. These last 24 columns provide/represent plasma drug concentrations of individual subjects reflecting variations in both lag time and bioavailability. These data may be used to draw C-t profiles for individual subjects and/or mean values as shown in Figure 2. Once the analyst has these individual values, the required bioavailability parameters C_{max}, T_{max}, AUC, along with their SD values can easily be calculated which may be used for potential bioavailability assessment of the product or to compare with from another product/batch.

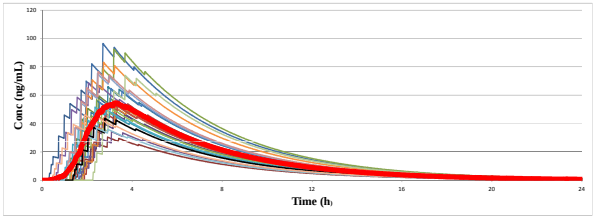

Figure 2: C-t profiles obtained/simulated from drug dissolution profiles of a 60 mg IR tablet product. Thin lines represent results for individual subjects while the thick line represent mean values for each sampling time.

For clarity of explaining the introduction of the lag time and calculating the plasma concentrations, a partial worksheet is provided (Link, please view it with at least 4x magnification). Data is shown for sampling times for up to 150 minutes, to reduce the size of the attached file, however, the figures shown here are based on data up to 24 hours.

The values (±SD) for bioavailability parameters obtained from this exercise representing/simulating a 24-subject 60-mg diltiazem IR tablet product are as follows: AUC (346±101 ng·h/mL); C_{max} (59.5±17.3 ng/mL);T_{max}(2.7±0.5 h).

Next, if the objective is to conduct a different study for comparison with the previous one, all one has to do is to run a dissolution test for the other product, convert the dissolution results into C-t profiles and calculate the bioavailability parameters from these profiles exactly like is done earlier. To demonstrate this step, the following represent the values (±SD) for bioavailability parameters obtained from a simulated 24-subject 120-mg diltiazem ER capsule product: AUC (611±167 ng·h/mL); C_{max} (57.74±15.9 ng/mL);T_{max}(5.20±0.41 h). The dissolution study of the second product, and corresponding C-t profiles as shown in Figure 3 along with the bioavailability parameters, clearly show that product is a slow release or an extended release type product. It is important to note that lag-time is a human subject characteristic while F is a drug property, however, both are independent of the product/formulation. Therefore, for both products the same lag-time and F values were used.

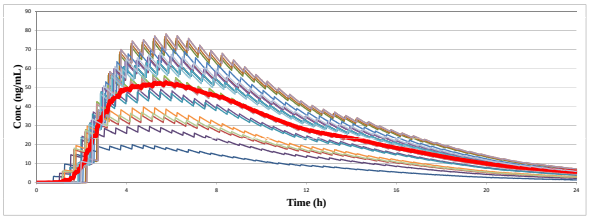

Figure 3: C-t profiles obtained/simulated from drug dissolution profiles of a 120 mg ER capsule product. Thin lines represent results for individual subjects while the thick line represent mean values for each sampling time.

The availability of SD values not only provides a more realistic representation of a human bioavailability study but also facilitate statistical analyses for comparison purposes.

Although, in this example the second set of data is of an ER product but the data can be from any type of product such as from different batches, product having different formulations or from another product (e.g. generic). The methodology of estimation of C-t profiles and corresponding bioavailability parameter will remain exactly the same. For example, a "simulated" dissolution study is described showing profiles of three different IR diltiazem products (Figure 4) demonstrating different dissolution release characteristics. These profiles represent products having faster, normal

(reference) and slower dissolution characteristics. It is clear from Table 1 that both faster and slower release characteristics are expected to be bioequivalent to the reference product based on the usual bioequivalency parameters (C_{max} and AUC, $p > 0.05$ for both). On the other hand, based on common methods of evaluation: there is more than 10% difference in dissolution results at different time points or analysis based on similarity factor, may yield a different conclusion (F2=51, a borderline case) that dissolution characteristics of the product may show bio-in-equivalencies.

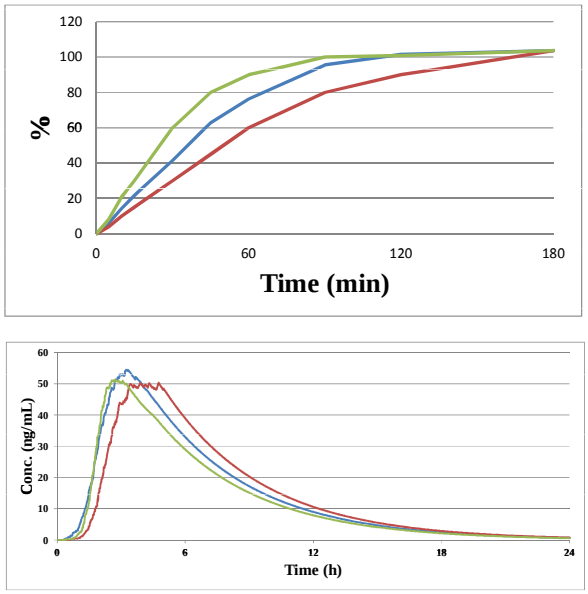

Figure 4: Top- Dissolution profiles of three products having faster, normal (reference) and slower drug release characteristics; Bottom - corresponding calculated C-t profiles using convolution technique.

It is important to note that different dissolution rates for the products produced respective rate of appearance of drug in vivo, however, C_{max} values did not match the dissolution rates which are often assumed in practice. That is, a faster dissolution rate is expected to provide higher C_{max} and vice versa. Therefore, for more appropriate interpretation of dissolution data one should evaluate and rely on predicted C-t rather than raw dissolution data i.e. percent dissolution with time.

An interesting observation here is that there is a significant difference ($p < 0.001$) in the T_{max} values for products; reference vs slower version but not ($p > 0.05$) for the reference vs faster version product. This observation is difficult to explain at present, however, it is generally accepted that the dissolution release up to a certain rate does not significantly impact absorption rate/or appearance in plasma. However, after a certain rate, the difference in absorption may be observed. Further, studies/assessments are needed to fully explain this behaviour, however, the technique may be used in defining and/or establishing this threshold.

Table 1: Mean (±SD) bioavailability parameter values calculated from C-t profiles for three products with simulated normal, faster and slower dissolution profiles of a 60-mg diltiazem IR product.

	C_{max} (ng/mL)	AUC (ng.h/mL)	T_{max} (h)
Normal	59.5±17.3	346±101	2.7±0.5
Faster	54.5±13.0	314±75	2.7±0.4
Slower	54.8±14.2	354±92	4.3±0.4*

*$p < 0.05$ for Reference vs Slower

The approach described here for comparing product characteristics based on predicted C-t profiles is simple and practical yet extremely powerful in evaluating products based on dissolution results with its physiological relevance. It appears that such an approach may indeed have a potential of genuinely reducing the number of human bioavailability studies, an extremely sought after feature at present for manufacturers of both generic and branded products.

Furthermore, the approach as described above using three products having different release characteristics can effectively be used for setting specifications/tolerances for dissolution testing. This approach of setting specification is superior, as it is linked to a potential physiological outcome, rather than the one currently followed based on only dissolution results.

The following summarizes the observations and their interpretations:

1. Dissolution results/profiles can be converted to show a typical human bioavailability outcome.

2. The inter-subject variability as observed in human bioavailability studies can be simulated from dissolution results by incorporating stomach emptying time and bioavailability factor.

3. As an analyst can obtain associated variabilities (variance or standard deviation) for the calculated bioavailability parameters, appropriate statistical analysis (*t–test*, ANOVA) can be performed to establish bio(in)equivalency of the products.

4. All the calculations to include stomach emptying time, bioavailability factor and their respective variabilities along with randomization can be performed using Excel spreadsheet software.

5. The methodology provides a simple approach for comparing dissolution results using C-t profiles based on typical bioavailability parameters such as AUC, $C_{max,}$ and $T_{max.}$ This makes the evaluation of dissolution results physiologically relevant, thus providing a superior approach compared to currently used empirical parameters such as *Q, F2 e*tc which have no physiological link or relevance.

6. This approach provides a more appropriate and physiologically relevant means for setting specifications for dissolution testing.