

Determining blood concentration-time (C-t) profiles from in vitro dissolution results and product evaluation – carbamazepine.

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Summary:

Using a recently suggested approach, based on IVIVC principles, C-t profiles (or blood levels) are determined for different strengths and release types (IR and ER) of carbamazepine products from in vitro drug dissolution results. Drug dissolution tests were conducted using the crescent-shaped spindle (25 rpm) with 900 mL of water containing 0.5% of SLS as dissolution medium. Predicted blood levels along with the derived pharmacokinetic parameters (T_{max} , C_{max} , and AUC) compare remarkably well with the corresponding human in vivo values reported in the literature. It appears that the approach described previously, and further detailed here, provides a powerful analytical technique for predicting blood levels and then evaluating product quality by establishing their equivalencies.

Introduction:

In vitro drug dissolution/release tests are conducted to estimate or predict in vivo drug release characteristics of a product. Direct estimation of in vivo drug dissolution is usually not possible and therefore blood drug concentration-time (C-t) profiles are used for this purpose. These C-t profiles which are obtained after administering drug products such as tablets and capsules in humans reflect the drug products' in vivo drug dissolution characteristics and hence their quality.

The practice/process of relating in vitro and in vivo's, dissolution results (profile) is commonly known as in vitro-in vivo correlation or IVIVC. It is important to note that IVIVC reflects the underlying principle or process but not the objective by itself for conducting a dissolution test. Determining the C-t profiles (or drug levels of a drug) is the objective of any drug dissolution testing.

It may be appropriate to provide a brief background discussion as to why a derived C-t profile should be an objective or requirement. A formulation developer may have a raw drug powder and wishes to formulate a product (tablet or capsule) of certain release characteristics considering its pharmacological response. The objective would then be to develop a formulation to achieve certain blood levels of the drug for an effective and safe product. That is, the formulator wishes to establish an appropriate C-t profile from different combinations of formulations prior to going to in vivo studies (assessments) in humans. Once a formulation is established, the product developer takes the formulation and bench-scale production attributes to large scale production attributes. The objective here is that by making the necessary changes in formulation and/or manufacturing attributes, the product developer would like to maintain the desired blood-levels achieved by the formulator. Moving along with the manufacturing side, once the product is developed, quality control laboratory wishes to ascertain that batch to batch, including stability of the batches, quality remains consistent, that is, the product is capable of providing the same blood levels as established by the formulators and the product developers.

The critical aspect of the preceding discussion is that each and every stage of product development is focused on achieving the desired blood levels i.e., C-t profiles. However, the main tool available to the developers is usually an in vitro dissolution tester. Human in vivo studies (bioavailability/bioequivalence) are usually conducted as confirmatory and in most cases, such as for QC purposes, these are seldom conducted. Therefore, there is heavy reliance on dissolution tests for reflecting in vivo drug levels or C-t profiles.

The most direct way to achieve this objective is to derive expected blood-levels (C-t profile) from the drug dissolution results. Once the predicted blood profiles are obtained for the test products(s) they may be evaluated or compared as if these were

obtained from a human in vivo (bioavailability/bioequivalence) study to assess comparative qualities of the test vs reference products. The three most commonly used parameters in this regard (for profile evaluations) are area under the C-t profile (AUC), the peak concentration (C_{max}) of the drug in the profile and the time (T_{max}) the drug takes to reach C_{max} .

Unfortunately, the current practices of dissolution testing do not provide this critical link to C-t profiles or blood levels therefore the use and credibility of dissolution testing often faces serious questions.

Recently a simple methodology has been described to derive drug blood levels from dissolution results [1, [link](#)]. The suggested new approach of determining the C-t profiles from dissolution testing fills this gap. A description of the theoretical basis and the relevant calculation procedure for the suggested approach has been described previously [1].

There are significant advantages in using the suggested procedure. (1) The procedure does not require an in vivo (or bio-) study for the test products to obtain pharmacokinetic parameters (bioavailability factor, F, volume of distribution, V_d , elimination rate constant, k_e or equation), but values available in the literature may be used. This is essential at the product development stage where products are to be developed with expected in vivo behavior based on dissolution characteristics (2) The procedure is independent of the product type i.e. both IR and ER products can be analyzed using the same analytical technique and pharmacokinetic parameters. (3) It is not necessary to purchase sophisticated computer software since simple spreadsheet software may be used. (4) The technique is quite easy to automate so that when dissolution results are entered, one can see the outcome immediately as the C-t concentration and/or profiles.

This article describes an application of the suggested procedure for the evaluation different carbamazepine products.

Material and Methods:

Pharmaceutical Products: Immediate-release (IR) carbamazepine tablet (200 mg) products were obtained from the local Canadian market. The brands of these products are identified as A, B and C. Extended-release (ER) products of 200 and 400 mg strengths were also obtained from the local Canadian market and the brands are identified as D, E and F [2]. All these products are interchangeably prescribed because of the bioequivalency of the respective (release and strength) products [3].

All other chemicals and solvents were of analytical grade and used as supplied by the suppliers.

Stirring/Mixing Spindle: The [crescent-shape spindle](#) was used in all experiments. The spindle [4] is designed to fit into the currently used dissolution apparatuses as a substitute for the

currently employed paddle spindle. The agitator has a stem part and the lower half is curved to conform to the shape of the vessel in which it is rotated, but with no direct contact with the surface of the vessel.

The end of the stem conforming to the bottom part of the vessel has filamentary elements filling the gap between the stem and the bottom part of the vessel. Therefore, when the device attached to the vertical shaft and rotates, the brush-type agitator will sweep through the bottom and sides of the vessel accomplishing even distribution and mixing the disintegrating material thus avoiding accumulation (coning) [5].

Instrumentation: The dissolution tests were conducted as described previously described using a Vankel system (VK 700) which is comprised of a bath with six vessels and met the physical and mechanical specifications as required by the USP [6]. All dissolution tests were conducted using the crescent-shape spindle at a speed of 25 rpm.

Prior to the use, the dissolution media were equilibrated at 37 °C overnight to de-aerate the medium so that bubble formation during the test, due to escape of dissolved gases, was minimized.

The tests for carbamazepine products were conducted using 900 mL water containing 0.5% of sodium lauryl sulfate. The amount of carbamazepine dissolved in each vessel was determined at various time intervals; up to 3 hours for conventional release products and 24 hours for extended release products. The quantitation was done by ultraviolet absorbance at 288 nm of filtered portions of the solution being tested, in comparison with a reference solution having a known concentration of carbamazepine standard [7].

Data Analysis: The data were collated and analyzed using MS Excel Spreadsheet software.

Pharmacokinetic parameters: The pharmacokinetic parameters were obtained from the literature [8] and the values used were as follows, bioavailability=F=100%, Volume of distribution= $V_d=1.26$ L/kg, Body weight=70 kg, Elimination Half-life ($t_{1/2}$)=36.39 hr., Elimination rate constant= $k_e=0.000317$ min⁻¹.

Derivation of blood concentrations from dissolution results: The suggested procedure as described previously [1] is based on five steps: (1) Converting percent drug release values from a dissolution test into discrete amounts (doses, in mg etc) within every sampling time; (2) converting the discrete percent doses into amounts available in the blood by multiplying it with the drug's bioavailability factor, obtained from the literature (3) calculating decreasing amount of drug in blood with time, separately for every dose/amount segments, using the drug's elimination rate (or rate equation), obtained from the literature. (4) Adding all the calculated drug levels (amounts) for every time. (5) Dividing blood amount at every time by volume of distribution, value obtained from the literature, to calculate the blood concentration of the drug. This will provide the expected blood level profiles.

Results:

The drug dissolution profiles of all the nine products are shown in figures below. The predicted C-t profiles are given in the figures (4-6). To evaluate these C-t profiles, various pharmacokinetic (PK) parameters, C_{max} ($\mu\text{g/mL}$) T_{max} (hr) and the AUC ($\mu\text{g}\cdot\text{hr/mL}$), were calculated which are used for comparing with their corresponding in vivo values or between products themselves. The values for the derived PK parameters ${}_p\text{AUC}$

($\mu\text{g}\cdot\text{hr/mL}$), ${}_pC_{max}$ ($\mu\text{g/mL}$) and ${}_pT_{max}$ (hr) are provided in the tables. The preceding subscript "_p" is added to indicate that these PK parameters represent predicted values obtained from the in vitro dissolution testing, unlike the usual parameter symbols which are obtained from the in vivo bioavailability studies.

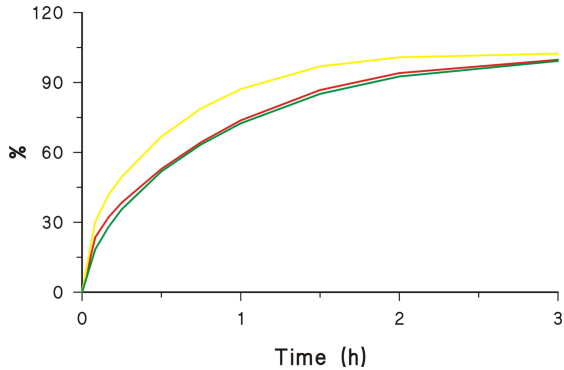


Figure 1: Mean (n=6) Percent drug release from three 200-mg IR carbamazepine products (A, B, C).

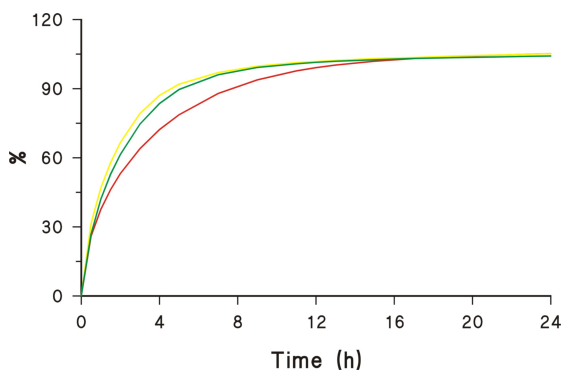


Figure 2: Mean (n=6) percent drug release from three 200-mg ER carbamazepine products (D, E, F).

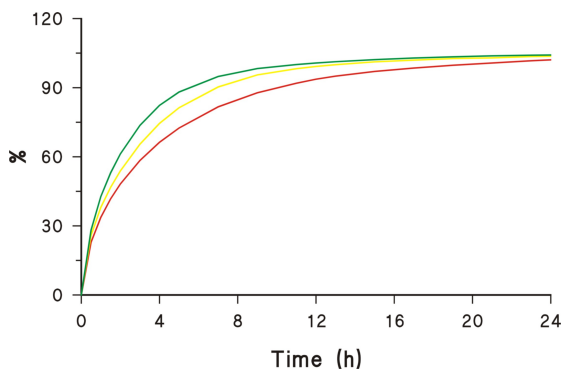


Figure 3: Mean (n=6) percent drug release from three 400-mg ER carbamazepine products (D, E, F).

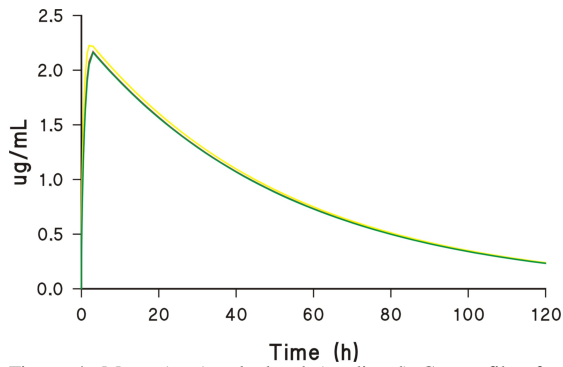


Figure 4: Mean (n=6) calculated (predicted) C-t profiles from dissolution results as shown in Figure 1.

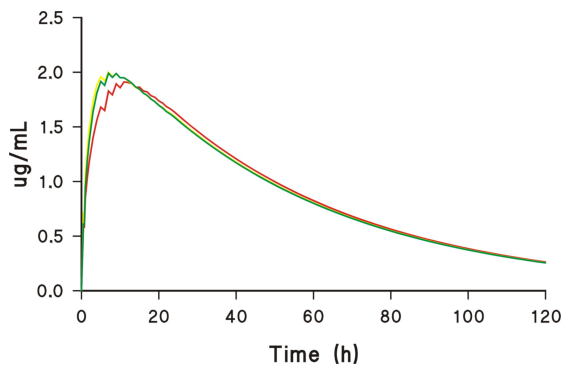


Figure 5: Mean (n=6) calculated (predicted) C-t profiles from dissolution results as shown in Figure 2.

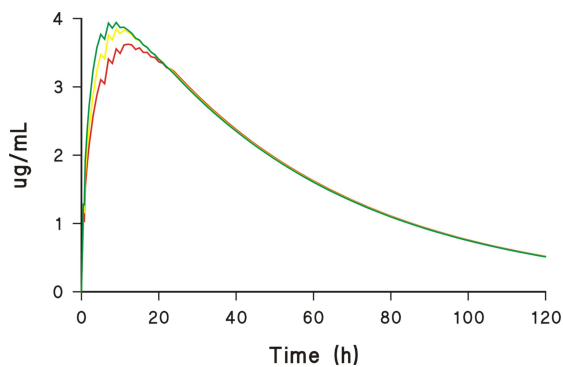


Figure 6: Mean (n=6) calculated (predicted) C-t profiles from dissolution results as shown in Figure 3.

Discussion:

The main objective in conducting a dissolution test is to predict or estimate in vivo drug release in humans. As the in vivo drug release is reflected by the C-t profiles, hence, the objective becomes relating (more accurately predicting) C-t profiles from in vitro dissolution results.

It becomes obvious from a quick review of the literature that there has been an intense research effort in the area of IVIVC with limited success (e.g see [10]). The reasons for this limited success appear to be: (1) inadequate simulation of in vivo environment for in vitro testing, e.g., commonly suggested dissolution apparatuses such as paddle and basket do not provide the stirring and mixing mechanism which is a critical process, within the in vivo environment; (2) hydrodynamics within dissolution vessels provide high variability in dissolution results which are usually not related to test products, thus making it difficult, if not

impossible, to characterize true quality of the test products; (3) lack of an objective and consistent approach for selecting experimental conditions e.g., dissolution medium, apparatus and/or operational parameters such as rpm; (4) experimental conditions are set based on expected or desired dissolution characteristics of test products, leading to product specific conditions rather than reflecting in vivo environment. For example, IR and ER products are often tested using different sets of experimental conditions whereas in vivo products are exposed to the same or common environment; (5) lack of emphasis on determining or predicting C-t profiles from in vitro results, which is, as stated above, the main objective of dissolution testing; (6) current practices usually require stand alone IVIVC studies which often mean conducting multiple bioavailability/ bioequivalence and in vitro dissolution studies to select the product specific experimental conditions for dissolution testing rather than evaluation of products.

Studies have demonstrated that if the tests are conducted using modified dissolution apparatuses which would provide efficient stirring and mixing environments, testing can become more relevant in the predicting products' characteristics and in particular for their in vivo behavior [10, 11]. One such modification is the use of a new stirrer, known as crescent-shaped spindle, for the vessel based apparatuses (paddle and basket). A number of reports are available in the literature demonstrating the advantages of using the vessel based apparatuses with the crescent-shaped spindle. In a recent report, where a simple and practical approach has been described for obtaining C-t profiles, it has been shown that results obtained with crescent-shaped spindle appears to predict blood levels quite accurately.

This article further elaborates the principles as described in that publication and demonstrates how the methodology may be used in product evaluation based on predicted C-t profiles.

As described in the methodology section above for the suggested approach one starts with the dissolution profiles, calculates discrete amounts of drug release between dissolution sampling intervals and combine these with pharmacokinetic parameters (obtained from the literature) using simple arithmetic calculations (using spreadsheet software), enabling one to obtain blood levels or C-t profiles of the drugs.

In this article, drug dissolution data is used from the study previously described in the literature [2], where dissolution tests are conducted for different products, nine in total, having different strengths and release types (IR and ER). Drug dissolution profiles of the products with corresponding C-t profiles are given in figures 1 to 6. The derived or predicted pharmacokinetics parameters are provided in the Table 1.

Once the C-t profiles are obtained, they may be used in a number of different ways, depending on the purpose and need. For the purpose of this article the first objective is to see if the procedure is indeed capable of providing expected blood levels. Usually two parameters are commonly referred to in this regard, which are C_{max} and AUC, reflecting rate and extent of drug absorption, respectively. In comparison to results reported in the literature, all the nine products tested provide accurate prediction of blood levels. Note that the in vivo values are twice those obtained from in vitro dissolution values for 200-mg strength tablets. The reason for this is that the dose for the in vivo study was twice as much (2 tablets of 200-mg) thus the parameter values should be twice those of the single tablet value. Therefore, it is safe to conclude that indeed the methodology of obtaining blood levels or C-t profiles, as suggested, appears validated and applicable.

Another way one would use the C-t profiles is to compare equivalency of the products. The equivalency evaluation perhaps

is the major use of dissolution testing, which extends to product development, modification/improvement and quality control/assurance. For this purpose, parameters (C_{max} and AUC) are evaluated to establish the sameness of the C-t profiles and

thus the products. As noted in the in methodology section, as these products are interchangeably prescribed, thus are equivalent in vivo. In this regard, the derived pharmacokinetic parameters predicted their equivalency very well indeed.

Table 1: Pharmacokinetic parameters values for predicted C-t profiles along with corresponding in vivo values from the literature.

	<i>In Vitro</i>				<i>In Vivo</i> ¶	
	200-mg IR Mean±SD	200-mg ER Mean±SD	400-mg ER Mean±SD		200-mg IR§ Mean±SD	400-mg ER Mean±SD
pC_{max}	2.23±0.03 (A)	1.99±0.02(D)	3.94±0.03(D)	C_{max}	4.74±1.27	3.88±0.90
pT_{max}	2.33±0.52 (A)	8.00±1.10(D)	8.33±1.03(D)	T_{max}	8.6±2.8	14.7±7.2
$pAUC$	109.66±1.19(A)	111.49±0.81(D)	223.09±2.56(D)	AUC	220.42 ±55.94	211.54±47.67
pC_{max}	2.17±0.02(B)	1.91±0.02(E)	3.85±0.05(E)	C_{max}	4.34±1.24	3.65±1.42
pT_{max}	3.00±0.00(B)	11.00±0.00(E)	9.00±0.00(E)	T_{max}	9.7±4.5	14.3±9.3
$pAUC$	106.75±1.14(B)	112.18/1.26(E)	221.30±2.87(E)	AUC	211.37 ±52.97	202.77±79.95
pC_{max}	2.16±0.03(C)	2.00±0.03(F)	3.62±0.02(F)			
pT_{max}	3.00±0.00(C)	7.33±0.82(F)	12.00±0.63(F)			
$pAUC$	106.38±1.62(C)	112.42±2.02(F)	217.88/2.12(F)			

p- Prefix reflects values obtained from the predicted C-t profiles. ¶ Literature values taken from ref. [8]. § 200-mg tablets were administered as 400-mg dose with 2 tablets per volunteer. Alphabet markings represent different manufacturers.

Thus, the predicted drug levels or C-t profiles appear to fulfill the requirements of IVIVC and provide expected drug levels in vivo, as well as equivalency of drug products, based on the derived pharmacokinetic parameters commonly used for in vivo testing.

Another parameter which is used in equivalency testing is the T_{max} . This parameter generally is known for its high variability, in particular in vivo, as is evident from the values in Table 1 as well. The reason for its high variability may be because of high variability in stomach emptying time, which not only depends on the physiological state of the stomach (empty or fasting, fed along with food type e.g. high fat or other wise, age, gender etc.) but also on the genetic makeup of the individual [12,13]

Further, since drug absorption generally occurs from small intestinal part, where drug is available after passing through stomach, there should always be a lag time in overall dissolution/absorption of drug and its availability in the blood. In vitro dissolution tests do not provide such a lag, therefore, in vitro peak concentration time would be shorter than in vivo by a difference of the lag time. If one corrects these in vivo emptying and lag times one would be able to get a fairly accurate estimation of in vivo T_{max} from the in vitro results. In this case, the lag time appears to be on average five and an half hour, which seems to be a fairly accurate reflection of commonly reported results in the literature [12, 13]. Further work to look into this aspect (T_{max}) in detail is in progress.

As stated above, the in vivo drug dissolution, and thus absorption depends on number of physiological variables, and therefore it is understandable that pharmacokinetic parameters will be highly variable as well. On the other hand, in vitro dissolution testing is usually conducted under fairly homogenous and controlled experimental conditions, and therefore, the expected variability in results would be minimal. This differential of in vitro and in vivo variabilities is apparent in the Table 1. An advantage of observing this difference in variabilities is that by using C-t profiles obtained from dissolution testing one would be able to clearly

establish the source of variability in C-t profiles from in vivo and/or in vitro (product quality). Under current practices, such separation of variabilities is not possible showing that there is a clear advantage of the approach based on deriving C-t profiles from in vitro dissolution results.

There appears to be a common belief in the scientific community that every time an IVIVC is to be established, a fresh in vivo (bioavailability) study will be needed. This is not an accurate belief and it originates from the use of apparatuses (e.g., paddle and basket) which, usually, do not provide appropriate simulation of in vivo environment. Thus, with each IVIVC study the analyst adjusts or selects the experimental conditions to match the in vivo results to achieve desired in vitro dissolution results. This would be similar in analogy of achieving a desired density value of a given liquid/solid by adjusting the weight and volumetric measuring scales. Obviously, it will be inaccurate. Similarly determining C-t profiles, drug dissolution testing experimental conditions should also be fixed and product independent.

This is, in fact, a unique ability of the approach of dissolution testing using crescent-shaped spindle in which a single set of experimental conditions are employed. This facilitates the development of true IVIVC. All the testing, as reported here, was conducted using a single set of experimental conditions, and then based on the suggested methodology C-t profiles are determined which results in expected in vivo plasma drug levels reported by a completely independent study.

Thus the use of the crescent-shaped spindle for dissolution testing, along with the newly suggested simple approach of calculating blood levels or C-t profiles, appears to provide a powerful analytical tool for pharmaceutical product evaluation.

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