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Original Article

# HYPOTHETICAL *IN VIVO* BEHAVIOR OF CARBAMAZEPINE TABLETS FROM *IN VITRO* RELEASE DATA OF USP APPARATUS II AND IV AND DISSOLUTION MEDIA OF PHYSIOLOGICAL RELEVANCE

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**ABSTRACT**

**Objective**: To estimate the hypothetical *in vivo* behavior of carbamazepine tablets (immediate-release, 200 mg) with dissolution data and a convolutional approach.

**Methods**: USP apparatus II and IV and media at pH 1.2, 4.5, and 6.8 (all containing 1% sodium lauryl sulfate) were used. The dissolved drug was calculated from 10 to 60 min. The dissolution profiles were compared with $f_2$ data and some dissolution parameters. *In vitro* release data were adjusted using several mathematical models. Predicted plasma levels were calculated using dissolution data and published pharmacokinetic information. A criterion for prediction error<10% for peak plasma concentration and area under the curve is considered suitable.

**Results**: After 60 min, with both USP apparatuses, all formulations released>75%. Similar dissolution profiles, with all formulations using USP apparatus II at pH 4.5 and 1.2 and with USP apparatus IV at pH 1.2, were found ($f_2$>50). In almost all the comparisons, dissolution parameters were statistically significant different (*P<0.05). Due to the diversity of the fitting results, no comparisons were made. Prediction errors<10% were found for all formulations using USP apparatus II at pH 4.5 and reference using USP apparatus IV at pH 6.8 and 4.5.

**Conclusion**: USP apparatus IV showed good discriminatory capacity; however, better *in vivo* predictions were obtained with USP apparatus II. Corroborating our findings from human studies using the formulations used is necessary.

**Keywords:** Carbamazepine, Convolution, Flow-through cell method, Multisource formulations, USP apparatus IV


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## INTRODUCTION

Carbamazepine is a widely used antiepileptic drug with narrow therapeutic range [1]. Overlaps between therapeutic and toxic doses have been consistently reported in the scientific literature [2]. The loss of seizure control and occurrence of adverse effects when one formulation is exchanged for another have been reported [3]. The carbamazepine biowaiver monograph resumes some important pharmacokinetic parameters such as 70-78% of bioavailability factor (f), 0.79-1.86 l/kg volume of distribution (Vd), and 34-38 h of plasma elimination half-life (t½) [2].

Dissolution studies are important for evaluating the *in vitro* release mechanisms of drugs, especially those with solubility problems. The conditions for the pharmacopeial dissolution test for carbamazepine tablets were the paddle method (USP Apparatus II) at 75 rpm and 900 ml of water containing 1% sodium lauryl sulfate (SLS). There are two tolerances 1) at 15 min, the dissolved amount should be 45-75%, and 2) at 60 min, not less than 75% should be dissolved [4]. A biowaiver monograph for carbamazepine immediate-release solid oral dosage forms has been published [2]. The monograph shows the risk of waiving *in vivo* bioequivalence studies by *in vitro* studies in the approval of new and/or reformulated carbamazepine drug products manufactured as immediate-release oral formulations.

Comparative *in vitro* dissolution tests when applying the Biopharmaceutics Classification System-based biowaiver approach should be conducted under several conditions, such as 1. Basket method (USP apparatus I) or USP apparatus II, 2. 900 ml or less volume of dissolution medium, 3. Temperature of dissolution medium 37±1 °C, 4. At least 12 units for each dissolution profile determination, 5. Three buffers were used: pH 1.2, pH 4.5, and pH 6.8 [5].

To better simulate the environment of the gastrointestinal tract inside the vessels, it is possible to use another type of dissolution media. Biorelevant dissolution media such as fasted-state simulated intestinal fluid (FaSSIF) or fed-state simulated intestinal fluid (FeSSIF) are complex mixtures of components (sodium taurocholate, lecithin, sodium phosphate, sodium chloride, pancreatin and sodium oleate) at various concentrations and pH levels [6]. However, these types of media are expensive and require special care when handling them. Dissolution studies on carbamazepine tablets with significant differences in dissolution performance, even within a single brand, have also been published [7].

USP apparatus I and II cannot mimic the hydrodynamic environment of the gastrointestinal tract [8]. To study the *in vitro* release behavior under these characteristics, the flow-through cell method (USP apparatus IV) was selected. The advantages of USP apparatus IV over other USP apparatuses have been reported [9]. The USP apparatus IV generates drug extraction similar to that of *in vivo* absorption [10]. Previous reports have documented a better discriminative capacity to test the *in vitro* release performance of carbamazepine tablets using USP apparatus IV than pharmacopeial conditions [11].

In contrast, the estimation of plasma concentration-time profiles is carried out using a mathematical approach called *convolution*. This technique is simple and provides a good platform for developing bioequivalent formulations during the product development stage. Based on the superposition principle, convolution is a model-independent method for computing *in vivo* absorption and modeling *in vitro-in vivo* data. *In vivo* pharmacokinetic parameters were predicted using drug release profiles as input function and pharmacokinetic parameters of reference formulation as weighted function [12].

The objective of the present study was to propose carbamazepine plasma concentration-time profiles using dissolution data from USP apparatus II and USP apparatus IV as well as dissolution media of physiological relevance (pH 1.2-6.8). The hypothetical pharmacokinetic parameters will be compared with the information from a previously published carbamazepine bioavailability study. The search for *in vitro* conditions that mimic *in vivo* performance will allow the development of better carbamazepine oral dosage forms.

## MATERIALS AND METHODS

### Chemicals

Five carbamazepine solid oral dosage forms (immediate-release tablets, 200 mg) acquired from the local market were used. The reference drug product Tegretol® (coded as R formulation) and four generic formulations (randomly coded as G1, G2, G3, and G4) of the same dose were tested. Methanol, hydrochloric acid, acetic acid, acetate, and phosphate salts were purchased from a local supplier (Xalostoc, Mexico). The carbamazepine standard compound was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Standard calibration curves were separately prepared in pH 6.8 phosphate buffer, pH 4.5 acetate buffer, and 0.1 N HCl (pH 1.2) to obtain final concentrations of 1.87-30 µg/ml. All solutions were prepared using 1% SLS.

### USP apparatus II

The in vitro release behavior of carbamazepine was determined using a USP apparatus II (Sotax AT-7 Smart Model, Switzerland) at 75 rpm. To quantify released carbamazepine, spectrophotometric determination (PerkinElmer spectrophotometer Lambda 35 Model, USA) was performed. The dissolved drug was calculated using standard calibration curves for each dissolution medium and the absorbance at 285 nm. Carbamazepine tablets were added on 900 ml of pH 6.8 phosphate buffer, pH 4.5 acetate buffer, and 0.1-N HCl (pH 1.2). All media were prepared using 1% SLS. The temperature of medium was 37.0±0.5 °C. The dissolved carbamazepine was quantified using filtered samples (nitrocellulose filters, Millipore) of the dissolution medium that were withdrawn at 10, 20, 30, 45, and 60 min (n=12).

### USP apparatus IV

Carbamazepine dissolution profiles were obtained using a USP apparatus IV (Sotax CE6 Model, Switzerland). The dissolution conditions were as follows: 22.6-mm cells, laminar flow at 16 ml/min, and the same dissolution media used with USP apparatus II. The dissolved drug was quantified using filtered samples of the dissolution medium that were withdrawn at 10, 20, 30, 45, and 60 min (n = 12). The percentage of dissolved carbamazepine was calculated using standard calibration curves at all pH values.

### Dissolution data analysis

The dissolution profiles of carbamazepine were compared (multi-source formulations vs. reference) with model-independent and model-dependent methods. The $f_2$ similarity factor was calculated as previously described [13]. For $f_2$=50–100, similar dissolution profiles were considered. In addition, the cumulative amount of drug released at the last sampling time ($Q_{60}$) and model-independent parameters mean dissolution time (MDT) and dissolution efficiency (DE) were calculated using the Excel add-in DDSolver [14]. Results were compared using a one-way analysis of variance (ANOVA) followed by a post hoc Dunnett's multiple comparison test with the support of SigmaPlot program (version 11.0). For model-dependent comparisons, dissolution data were fitted by several mathematical models such as: First-order, Higuchi, Korsmeyer-Peppas, Peppas-Sahlin, Makoid-Banakar, and Weibull models, and with the Excel add-in DDSolver [14]. The model with the highest adjusted determination coefficient ($R^2_{adjusted}$) and lowest Akaike Information Criterion (AIC) was selected as the best-fit model [15]. The *in-vitro* release mechanism of carbamazepine was also modeled using the hyperbola equation (y = ax/b+x) with the support of SigmaPlot (version 11.0). With a and b parameters time at which 50, 63.2, 75, and 85% of released dose ($t_{50\%}$, $t_{63.2\%}$, $t_{75\%}$, and $t_{85\%}$, respectively) were calculated and statistically compared. Statistically significant differences were considered when*P<0.05.

### Simulation of *in vivo* performance

Carbamazepine plasma concentration-time profiles were simulated using a simple numerical convolution method in MS Excel spreadsheet [16]. Because no actual carbamazepine plasma concentration-time profile was available, no deconvolution-based *in vitro*/*in vivo* correlation (IVIVC) approach was attempted. The *in vitro* release data from both USP apparatuses and published carbamazepine pharmacokinetic information, such as volume of distribution (Vd), bioavailability factor (f), and elimination rate constant (ke), were used to simulate plasma concentration-time profiles [2]. The hypothetical plasma levels of carbamazepine were fitted to a one-compartmental open model with first-order elimination using the Excel add-in PKSolver [17]. The pharmacokinetic modeling of carbamazepine was performed using this model [18]. The predicted peak plasma level ($C_{max}$) and area under the curve from zero time to infinity ($AUC_{0\text{-}inf}$) were calculated and compared with similar parameters observed in a carbamazepine bioavailability study [1]. The predictability of the convolution analysis was established by calculating the prediction error (%PE) for $C_{max}$ and $AUC_{0\text{-}inf}$. The %PE was calculated according to Eq.1 (values should not exceed 10%) [19].

$$\%PE = \frac{(observedvalue - predictedvalue)}{observedvalue} \times 100 \text{ Eq. [1]}$$

## RESULTS AND DISCUSSION

### Dissolution data

The dissolution profiles of carbamazepine in R, G1, G2, G3, and G4 formulations, obtained using both USP apparatuses and dissolution media of physiological relevance, are shown in fig. 1. As observed, all formulations showed different in vitro release performances, especially with USP apparatus IV, where the G1 formulation showed similar profiles in pH 6.8+1% SLS and 0.1-N HCl+1% SLS (f2>50). Using USP apparatus II, all formulations showed similar dissolution profiles with pH 4.5+1% SLS and 0.1-N HCl+1% SLS (f2>50). The f2 similarity factors were calculated because the coefficients of variation (CV) of all dissolution profiles met the international criteria (CV<20% at the earlier time points and<10% at other time points) [20].

These results suggest that the USP apparatus IV is a more discriminating method for differentiating the in vitro release performance of the multisource formulations since, under the same dissolution conditions, all generic drug products do not release the drug as the reference does. As a context, results in which the in vitro release of two-dose carbamazepine tablets (200 and 400 mg) were slower in the USP apparatus IV than in the USP Apparatus II have been reported [21]. The USP apparatus IV allows for a low-dissolution patron, which better reflects the *in vivo*. For orally administered drugs, *in vivo* dissolution is a prerequisite to ensure *in vivo* absorption [22].

### Model-independent comparisons

Parameters such as Q60, DE, and MDT for carbamazepine formulations calculated from dissolution data of USP apparatus II and IV are shown in tables 1 and 2, respectively. In almost all comparisons, statistically significant differences were found (*P<0.05).

Previously, the DE and MDT dissolution parameters were suggested for comparing the dissolution profiles [23, 24]. DE is defined as a parameter that relates the area under the curve to the total area of the rectangle formed by the theoretical 100% dissolution and the whole time of the test [25], whereas MDT is defined as the average time at which 63.2% of the dose is released [26]. In addition, MDT is a suitable value for establishing IVIVC level B. This level is related to MDT and mean residence time [27]. On the other hand, DE is a parameter that reflects dissolution performance, and it can be associated with an *in vivo* parameter, such as $C_{max}$ or $AUC_{0\text{-}inf}$. This type of association is the basis of IVIVC level C.

### Model-dependent comparisons

The comparison of dissolution profiles by model-dependent methods is not the first choice of analysis; usually, the model-independent f2 similarity factor is used. If this is not possible, a mathematical model can be fitted to the drug dissolution data, and the estimated model parameters can then be used to compare the dissolution profiles [28]. In the present study, the calculation of f2 factors was appropriate since the results did not exceed the permitted levels of variability, and the mathematical modeling proposed was carried out as an alternative to dissolution profiles comparisons. Kinetic modeling of in vitro carbamazepine release in USP apparatuses II and IV is shown in tables 3 and 4, respectively. As the number of parameters in each equation differs from the coefficient of regression, the R2adjusted and AIC were selected as parameters for evaluating the best fitting [22]. These only

provide a general overview of the model fit and do not provide any information about a mathematical model's performance in different sections of the drug release profile [28]. A specific kinetic model that describes the mechanism of carbamazepine release under the used experimental conditions was not found (all generic formulations and reference). Due to the variability in the adjustment results, comparison of dissolution profiles using model-dependent methods was not possible.

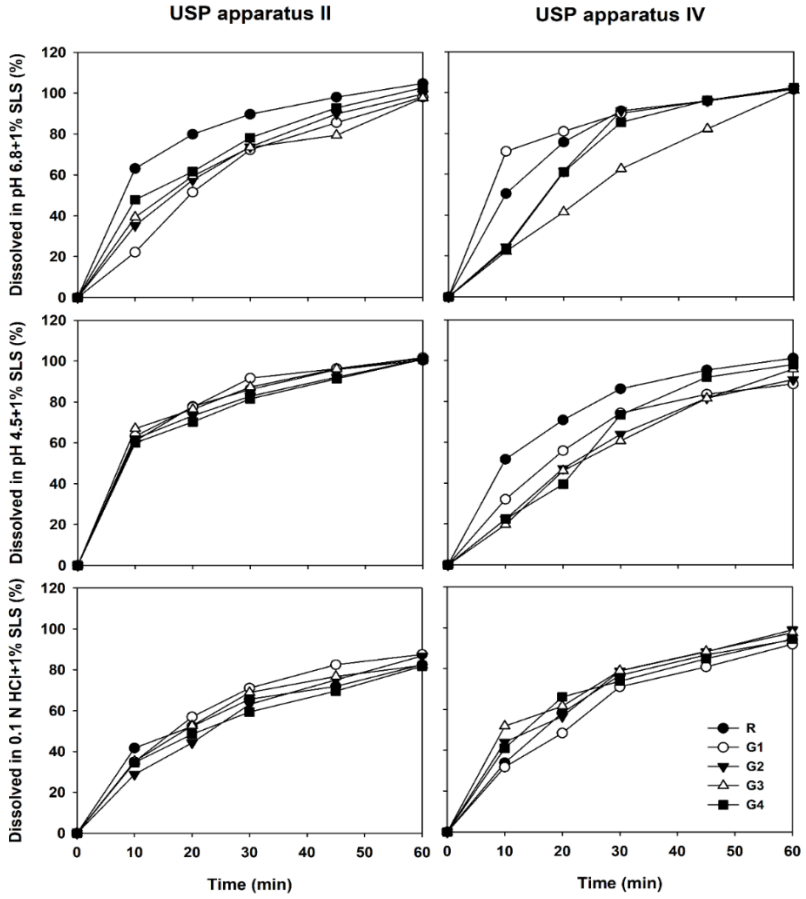


**Fig. 1: Dissolution profiles of carbamazepine of reference (R) and generic tablets (G1 – G4). For clarity, error bars were omitted, mean, n=12**

**Table 1: Dissolution parameters of carbamazepine tablets from data of USP apparatus II**

| Parameter | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| $f_2$ | – | 32.56 | 39.11 | 39.01 | 47.77 |
| $Q_{60}$ (%) | 104.77±0.92 | 98.12±0.86* | 99.60±0.40* | 97.69±0.63* | 102.68±1.06 |
| DE (%) | 80.90±1.37 | 61.00±0.38* | 65.74±0.30* | 63.84±0.47* | 70.58±0.91* |
| MDT (min) | 13.95±0.43 | 22.68±0.24* | 20.39±0.13* | 20.78±0.31* | 18.76±0.23* |
| $t_{50\%}$ (min) | 6.40±0.19 | 20.43±0.32* | 14.43±1.21* | 15.85±0.45* | 11.50±0.40* |
| $t_{63.2\%}$ (min) | 10.02±0.32 | 28.57±0.45* | 20.88±1.77* | 23.52±0.56* | 17.06±0.57* |
| $t_{75\%}$ (min) | 15.14±0.55 | 37.45±0.62* | 28.49±2.46* | 33.09±0.62* | 23.96±0.78* |
| $t_{85\%}$ (min) | 22.35±0.93 | 46.60±0.86* | 36.99±3.24* | 44.54±0.64* | 32.12±1.02* |
| | pH 4.5+1% SLS | | | | |
| $f_2$ | – | 78.43 | 76.39 | 77.66 | 68.77 |
| $Q_{60}$ (%) | 100.42±0.35 | 101.69±0.24 | 100.98±0.37 | 101.23±0.43 | 100.88±0.37 |
| DE (%) | 77.59±0.13 | 79.36±0.36* | 75.46±0.23* | 78.79±0.35 | 74.13±0.76* |
| MDT (min) | 13.63±0.15 | 13.17±0.28 | 15.16±0.13 | 13.30±0.16 | 15.90±0.45 |
| $t_{50\%}$ (min) | 6.90±0.10 | 6.32±0.11 | 7.33±0.09 | 6.19±0.08 | 9.07±0.39* |
| $t_{63.2\%}$ (min) | 10.95±0.15 | 9.97±0.17 | 11.73±0.14* | 9.93±0.12 | 14.27±0.57* |
| $t_{75\%}$ (min) | 16.83±0.23 | 15.20±0.25 | 18.28±0.25* | 15.53±0.18 | 21.69±0.76* |
| $t_{85\%}$ (min) | 25.42±0.34 | 22.71±0.34 | 28.20±0.47* | 24.11±0.31 | 32.32±0.91* |
| | 0.1-N HCl+1% SLS | | | | |
| $f_2$ | – | 59.97 | 58.55 | 71.58 | 67.84 |
| $Q_{60}$ (%) | 82.50±1.09 | 87.50±0.46* | 86.81±0.47* | 82.17±0.65 | 81.75±0.58 |
| DE (%) | 57.61±0.69 | 61.67±0.32* | 54.92±0.35* | 58.42±0.28 | 53.81±0.38* |
| MDT (min) | 18.09±0.25 | 17.69±0.34 | 22.04±0.23* | 17.32±0.26 | 20.49±0.32* |
| $t_{50\%}$ (min) | 17.11±0.40 | 15.26±0.14* | 22.54±0.33* | 17.55±0.20 | 20.24±0.47* |
| $t_{63.2\%}$ (min) | 29.63±0.69 | 23.56±0.21* | 32.76±0.48* | 28.15±0.35 | 32.47±0.75* |
| $t_{75\%}$ (min) | 52.72±1.62 | 34.85±0.34* | 44.87±0.68* | 44.00±0.65* | 50.84±1.39 |
| $t_{85\%}$ (min) | 105.59±6.58 | 49.93±0.59* | 58.50±0.93* | 68.22±1.32* | 79.46±3.18* |

Mean±SEM, n = 12. *P<0.05

**Table 2: Dissolution parameters of carbamazepine tablets from data of USP apparatus IV**

| Parameter | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| $f_2$ | – | 52.86 | 45.70 | 32.64 | 44.70 |
| $Q_{60}$ (%) | 101.56±0.20 | 101.79±0.19 | 101.84±0.39 | 101.09±0.35 | 102.41±0.35 |
| DE (%) | 76.68±0.10 | 80.77±0.07* | 69.99±0.12* | 56.87±0.42* | 68.77±0.25* |
| MDT (min) | 14.70±0.07 | 12.39±0.08* | 18.76±0.15* | 26.24±0.23* | 19.71±0.09* |
| $t_{50\%}$ (min) | 9.29±0.08 | 4.80±0.10* | 15.28±0.18* | 28.86±0.37* | 15.88±0.47* |
| $t_{63.2\%}$ (min) | 14.11±0.14 | 7.80±0.16* | 21.50±0.26* | 31.60±0.45* | 22.27±0.66* |
| $t_{75\%}$ (min) | 20.44±0.23 | 12.45±0.25* | 28.40±0.34* | 39.19±0.51* | 29.29±0.88* |
| $t_{85\%}$ (min) | 28.47±0.39 | 19.94±0.39* | 35.59±0.43* | 46.17±0.57* | 36.55±1.10* |
| | pH 4.5+1% SLS | | | | |
| $f_2$ | – | 43.86 | 35.77 | 34.40 | 36.70 |
| $Q_{60}$ (%) | 101.16±0.28 | 88.53±1.13* | 90.69±1.45* | 95.82±1.38* | 98.06±0.70* |
| DE (%) | 74.87±0.15 | 66.89±0.96* | 56.64±0.57* | 55.93±0.56* | 60.82±0.43* |
| MDT (min) | 15.59±0.11 | 11.00±0.26* | 22.42±0.70* | 24.94±0.32* | 22.78±0.17* |
| $t_{50\%}$ (min) | 9.74±0.12 | 16.40±0.62* | 22.38±0.54* | 23.63±0.33* | 21.46±0.41* |
| $t_{63.2\%}$ (min) | 14.93±0.21 | 24.54±0.94* | 31.54±0.56* | 32.34±0.55* | 29.06±0.49* |
| $t_{75\%}$ (min) | 21.88±0.37 | 34.84±1.35* | 41.80±0.64* | 41.48±0.89* | 36.85±0.53* |
| $t_{85\%}$ (min) | 30.97±0.64 | 43.37±1.86* | 52.69±1.00* | 50.48±1.31* | 44.34±0.56* |
| | 0.1-N HCl+1% SLS | | | | |
| $f_2$ | – | 63.12 | 65.48 | 55.54 | 66.71 |
| $Q_{60}$ (%) | 94.04±0.79 | 91.90±0.72 | 98.95±0.83* | 97.49±0.67* | 94.44±0.52 |
| DE (%) | 64.75±0.48 | 59.85±0.36* | 67.60±0.44* | 69.61±0.71* | 66.21±0.33 |
| MDT (min) | 18.68±0.30 | 20.91±0.27* | 18.99±0.30 | 17.16±0.35 | 17.93±0.19 |
| $t_{50\%}$ (min) | 16.25±0.41 | 18.79±0.26* | 13.98±0.23* | 11.86±0.27* | 12.77±0.19* |
| $t_{63.2\%}$ (min) | 23.60±0.65 | 27.32±0.33* | 20.92±0.29* | 18.39±0.44* | 19.74±0.29* |
| $t_{75\%}$ (min) | 32.32±0.99 | 37.48±0.46* | 29.71±0.36* | 27.37±0.71* | 29.26±0.44* |
| $t_{85\%}$ (min) | 42.15±1.45 | 48.98±0.77* | 40.44±0.54 | 39.58±1.16 | 42.08±0.71* |

Mean±SEM, n = 12. *P<0.05

**Table 3: $R^2$ adjusted/AIC data of carbamazepine tablets from data of USP apparatus II calculated to choose the best-fit model. Reference (R) and generic formulations (G1−G4)**

| Model | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| First-order | 0.984/28 | 0.970/33 | 0.990/26 | 0.984/28 | 0.980/30 |
| Higuchi | 0.914/40 | 0.950/37 | 0.990/26 | 0.988/27 | 0.989/25 |
| Korsmeyer-Peppas | 0.994/23 | 0.968/35 | 0.991/26 | 0.988/27 | 0.994/23 |
| Peppas-Sahlin | 0.997/18 | 0.992/25 | 0.998/15 | 0.986/29 | 0.994/19 |
| Makoid-Banakar | 0.997/17 | 0.990/27 | 0.998/15 | 0.986/28 | 0.995/18 |
| Weibull | 0.996/11 | 0.993/21 | 0.997/16 | 0.982/30 | 0.990/22 |
| | pH 4.5+1% SLS | | | | |
| First-order | 0.931/22 | 0.927/22 | 0.815/27 | 0.793/27 | 0.821/27 |
| Higuchi | 0.413/33 | 0.673/34 | 0.469/33 | 0.432/35 | 0.550/31 |
| Korsmeyer-Peppas | 0.976/17 | 0.943/22 | 0.994/10 | 0.979/15 | 0.988/13 |
| Peppas-Sahlin | 0.994/9 | 0.969/18 | 0.993/10 | 0.972/16 | 0.984/14 |
| Makoid-Banakar | 0.994/9 | 0.970/18 | 0.996/7 | 0.976/15 | 0.989/11 |
| Weibull | 0.994/8 | 0.954/19 | 0.997/6 | 0.965/15 | 0.981/1 |
| | 0.1-N HCl+1% SLS | | | | |
| First-order | 0.943/34 | 0.993/21 | 0.995/20 | 0.979/29 | 0.971/30 |
| Higuchi | 0.976/29 | 0.982/28 | 0.983/28 | 0.980/28 | 0.994/18 |
| Korsmeyer-Peppas | 0.993/21 | 0.981/29 | 0.991/25 | 0.982/28 | 0.996/15 |
| Peppas-Sahlin | 0.992/22 | 0.998/12 | 0.993/22 | 0.995/18 | 0.996/15 |
| Makoid-Banakar | 0.992/22 | 0.999/9 | 0.994/22 | 0.995/18 | 0.997/13 |
| Weibull | 0.988/25 | 0.999/10 | 0.991/24 | 0.993/21 | 0.994/14 |

Mean, n = 12

Dissolution rate parameters, such as $t_{50\%}$, $t_{63.2\%}$, $t_{75\%}$, and $t_{85\%}$, calculated using *in vitro* release data of USP apparatus II and IV are shown in tables 1 and 2, respectively. With the G1 and G3 formulations, tested with USP apparatus II and pH 4.5+1% SLS, no statistically significant differences in all dissolution rate parameters were found (*P>0.05), whereas with USP apparatus IV, all parameters of all formulations showed significant differences (*P<0.05). These results suggest that the drug-release mechanism is highly dependent on the hydrodynamic environment of the equipment, the acidity of the dissolution medium, and the formulation (excipients and/or manufacturing process).

**Hypothetical plasma concentrations**

After applying the convolution approach and adjusting the predicted carbamazepine plasma concentration-time profiles to a compartment model, the main pharmacokinetic parameters were calculated. Simulated $C_{max}$ and $AUC_{0\text{-}inf}$ were associated with human data [1] to calculate PE values. Results are shown in tables 5 and 6.

**Table 4: $R^2_{adjusted}$/AIC data of carbamazepine tablets from data of USP apparatus IV calculated to choose the best-fit model. Reference (R) and generic formulations (G1 – G4)**

| Model | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| First-order | 0.988/16 | 0.771/26 | 0.882/32 | 0.916/30 | 0.899/32 |
| Higuchi | 0.767/31 | 0.776/36 | 0.843/34 | 0.881/32 | 0.871/33 |
| Korsmeyer-Peppas | 0.895/28 | 0.993/8 | 0.806/36 | 0.989/19 | 0.855/34 |
| Peppas-Sahlin | 0.976/20 | 0.992/9 | 0.926/31 | 0.993/14 | 0.953/28 |
| Makoid-Banakar | 0.981/19 | 0.992/9 | 0.962/27 | 0.994/13 | 0.979/24 |
| Weibull | 0.982/17 | 0.987/10 | 0.981/22 | 0.991/14 | 0.994/15 |
| | pH 4.5+1% SLS | | | | |
| First-order | 0.981/18 | 0.963/22 | 0.961/25 | 0.941/28 | 0.885/33 |
| Higuchi | 0.844/29 | 0.917/27 | 0.906/30 | 0.890/31 | 0.846/34 |
| Korsmeyer-Peppas | 0.960/23 | 0.892/29 | 0.943/27 | 0.972/25 | 0.904/32 |
| Peppas-Sahlin | 0.993/12 | 0.961/23 | 0.987/17 | 0.991/17 | 0.913/31 |
| Makoid-Banakar | 0.994/12 | 0.965/22 | 0989/17 | 0987/20 | 0.958/27 |
| Weibull | 0.992/12 | 0.966/19 | 0.988/15 | 0.988/16 | 0.959/26 |
| | 0.1-N HCl+1% SLS | | | | |
| First-order | 0.984/19 | 0.980/20 | 0.953/24 | 0.894/26 | 0.969/21 |
| Higuchi | 0.942/26 | 0.957/24 | 0.962/23 | 0.860/27 | 0.928/25 |
| Korsmeyer-Peppas | 0.926/28 | 0.957/25 | 0.962/23 | 0.964/20 | 0.954/24 |
| Peppas-Sahlin | 0.977/21 | 0.971/23 | 0.954/24 | 0.953/22 | 0.966/22 |
| Makoid-Banakar | 0.981/20 | 0.971/22 | 0.954/24 | 0.955/21 | 0.967/21 |
| Weibull | 0.984/15 | 0.950/24 | 0.927/25 | 0.926/22 | 0.979/16 |

Mean, n = 12

**Table 5: Hypothetical $C_{max}$ and $AUC_{0\text{-}inf}$ and PE value for each parameter from data of USP apparatus II**

| Hypothetical value | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 2.49 | 2.39 | 2.42 | 2.34 | 2.47 |
| PE for $C_{max}$ (%) | -10.09 | -5.67 | -6.74 | -3.23 | -9.14 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 157.21 | 109.92 | 118.50 | 128.86 | 132.28 |
| PE for $AUC_{0\text{-}inf}$ | -6.27 | 25.69 | 19.89 | 12.88 | 10.57 |
| | pH 4.5+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 2.40 | 2.43 | 2.39 | 2.40 | 2.39 |
| PE for $C_{max}$ (%) | -5.89 | -7.32 | -5.31 | -5.96 | -5.42 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 147.50 | 148.63 | 160.08 | 158.70 | 155.42 |
| PE for $AUC_{0\text{-}inf}$ | 0.28 | -0.47 | -8.22 | -7.28 | -5.06 |
| | 0.1-N HCl+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 1.97 | 2.13 | 2.11 | 1.99 | 1.96 |
| PE for $C_{max}$ (%) | 13.07 | 6.09 | 7.00 | 11.99 | 13.33 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 115.16 | 106.61 | 101.40 | 101.93 | 105.45 |
| PE for $AUC_{0\text{-}inf}$ | 22.15 | 27.92 | 31.45 | 31.09 | 28.71 |

**Table 6: Hypothetical $C_{max}$ and $AUC_{0\text{-}inf}$ and PE value for each parameter from data of USP apparatus IV**

| Hypothetical value | R | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | pH 6.8+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 2.46 | 2.40 | 2.51 | 2.46 | 2.52 |
| PE for $C_{max}$ (%) | -8.40 | -6.09 | -10.70 | -8.54 | -11.11 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 133.48 | 168.14 | 112.62 | 109.88 | 113.35 |
| PE for $AUC_{0\text{-}inf}$ | 9.77 | -13.66 | 23.89 | 25.75 | 23.37 |
| | pH 4.5+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 2.44 | 2.16 | 2.21 | 2.33 | 2.41 |
| PE for $C_{max}$ (%) | -0.52 | 10.89 | 8.65 | 3.73 | 0.53 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 134.82 | 104.66 | 100.80 | 104.95 | 103.48 |
| PE for $AUC_{0\text{-}inf}$ | 6.38 | 27.31 | 29.99 | 27.11 | 28.13 |
| | 0.1-N HCl+1% SLS | | | | |
| $C_{max}$ (µg/ml) | 2.29 | 2.23 | 2.39 | 2.33 | 2.27 |
| PE for $C_{max}$ (%) | 5.63 | 7.96 | 1.53 | 3.77 | 6.41 |
| $AUC_{0\text{-}inf}$ (µgh/ml) | 112.57 | 108.29 | 125.00 | 134.35 | 125.33 |
| PE for $AUC_{0\text{-}inf}$ | 21.82 | 24.79 | 13.19 | 6.69 | 12.95 |

With USP apparatus II and dissolution medium of pH 4.5+1% SLS, both pharmacokinetic parameters of all formulations showed PE<10%. With USP apparatus IV, only hypothetical $C_{max}$ and $AUC_{0\text{-}inf}$ of the R formulation, tested in pH 6.8+1% SLS and pH 4.5+1% SLS; and G3 formulation, in 0.1-N HCl+1% SLS, showed PE values<10%.

Almost all hypothetical $C_{max}$ and $AUC_{0\text{-}inf}$ values agreed with *in vivo* data. $C_{max}$ values ranging from 1.15 to 2.70 µg/ml and AUCs ranging from 120 to 190 µgh/ml were observed after the administration of 200 mg of carbamazepine immediate-release tablets to healthy subjects [2].

USP apparatus IV has been documented as a useful element for establishing a meaningful correlation with low-solubility drugs; however, especially for carbamazepine, there are reports of significant IVIVC using USP apparatus I [29] and II [1, 30, 31]. Kovacevic *et al.* stated that despite the correlation between *in vitro* and *in vivo* data, the predicted plasma concentration profiles were insensitive to the differences in drug input kinetics. Such data are in accordance with the literature reporting that carbamazepine drug products with different dissolution kinetics were bioequivalent. Recently, authors of the carbamazepine biowaiver monograph have stated that pharmacopeial dissolution conditions appear to be useful not only for quality control purposes but also as a promising way forward to establishing meaningful IVIVCs for solid oral immediate-release dosage forms [2].

Several authors have stated that good predictions of plasma profiles from *in vitro* dissolution data of carbamazepine formulations have been achieved using pharmacopeial conditions (USP paddle apparatus and distilled water containing 1% SLS) [2]. The reported average pH of distilled water is 5.5 by pH-indicator strips and 5.73 by pH meter [32]. Our findings are supported by this information, as with all formulations, better predictions were obtained using USP apparatus II and a dissolution medium of pH 4.5+1% SLS. The convolutional approach used in this study is an option for testing the biopharmaceutical quality of carbamazepine products without simultaneous human studies. Despite this, the evidence of carbamazepine formulations failing to meet bioequivalence requirements suggests that the risk of a carbamazepine product failing the bioequivalence test is high [2].

Other researchers have reported that virtual bioequivalence offers an alternative approach that leverages computational methods to predict the bioequivalence of generic drugs without the need for extensive human studies. This involves developing and applying mathematical models that simulate the human body's drug absorption, distribution, metabolism, and excretion processes [33]. Sarkar *et al.* commented that *in vivo* predictive dissolution methods are considerably different from and more complex than USP quality control methodologies. An *in vivo* predictive method is considered a surrogate for forecasting *in vivo* drug release and potentially reduces the number of bioavailability/bioequivalence studies required [34].

Dissolution testing is an *in vitro* test used to assess and estimate the *in vivo* behavior of oral solid dosage forms. For drug product development purposes, dissolution tests are intended as an *in vitro* indicator of the *in vivo* performance of the dosage form. Ideally, dissolution data can be used to establish IVIVCs with clinically observed plasma-time curves. This can be achieved using computer-based models. In these models, the *in vitro* data were used as input functions, and the software used convolution algorithms to estimate the plasma-time curves observed *in vivo* [35]. In *in vivo* bioequivalence studies, the pivotal pharmacokinetic parameters $C_{max}$ and AUC are generally used to assess the rate and extent of drug absorption. On the other hand, the biopharmaceutics classification system (BCS)-based biowaiver approach is intended to reduce the need for *in vivo* bioequivalence studies, i. e., it can provide a surrogate for *in vivo* bioequivalence [5]. The accepted parameter to validate the estimated *in vivo* predictions is the calculation of the percentage of PE for $C_{max}$ and AUC. A PE value of 10% or less confirms the predictability of the proposed convolutional model. A value between 10% and 20% indicates inconclusive predictability and requires additional data. A PE ≥ 20% indicates inadequate or insufficient predictability. The $C_{max}$, $T_{max}$, and AUC can be used as surrogates for *in vivo* bioequivalence [19].

The results suggest that under the above-mentioned dissolution conditions, it is possible to evaluate generic drug products because the dissolution data of the reference formulation can be mathematically transformed into plasma profiles similar to those observed in a bioavailability study in humans, which generates information for safe interchangeability between carbamazepine formulations. Similar results were observed for ibuprofen soft gelatin capsules [36] and indomethacin hard capsules [37], both Class II drugs.

## CONCLUSION

Hypothetical human behavior of carbamazepine tablets was determined using *in vitro* release data of USP apparatus II and IV, dissolution medium of physiological relevance, and published pharmacokinetic information. Both USP apparatuses generate dissolution conditions in which the reference drug product simulates the *in vivo* performance of carbamazepine and is comparable to that observed. These conditions are the optimal settings to evaluate the rate and extent of *in vitro* release of carbamazepine multisource formulations. To facilitate the interchangeability of generic drug products, it is important to use in silico methodologies to optimize the design of low-cost pharmaceutical dosage forms that present the same therapeutic effect as the reference formulation. Given the variability in the *in vitro* results and *in vivo* proposals, further research is needed.

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Nil

## AUTHORS CONTRIBUTIONS

FD Reyes-Ramírez and JR Medina-López conceived and designed the experiments; YA Vera-Ángeles and FD Reyes-Ramírez performed the experiments; YA Vera-Ángeles and FD Reyes-Ramírez contributed to drug simulations; FD Reyes-Ramírez and JR Medina-López carried out the statistical analysis; YA Vera-Ángeles searched bibliographic data; FD Reyes-Ramírez and JR Medina-López wrote the paper. All authors have read and agreed to the published version of the manuscript.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest

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