Pharmaceutical Research (2023) 40:2167–2175
https://doi.org/10.1007/s11095-023-03578-x

ORIGINAL RESEARCH ARTICLE

# Revamping Biopharmaceutics-Pharmacokinetics with Scientific and Regulatory Implications for Oral Drug Absorption

**Nikolaos Alimpertis[1,2] · Athanasios A. Tsekouras[2,3] · Panos Macheras[1,2]**

Received: 11 May 2023 / Accepted: 25 July 2023 / Published online: 3 August 2023


## Abstract

**Purpose** The Wagner-Nelson and Loo-Riegelman methods developed in the 1960s and used since for the construction of percent of drug absorbed as a function of time as well as in *in vitro in vivo* correlations are re-considered in the light of the physiologically sound Finite Absorption Time (F.A.T.) concept developed recently.

**Methods** The classical equations for the percentage of drug absorption as a function of time were modified by taking into account the termination of drug absorption at F.A.T., replacing the parameters associated with the assumption of infinite drug absorption.

**Results** Mathematical analysis using the relevant Physiologically Based Pharmacokinetic Finite Time (PBFTK) models assuming one- or two-compartment drug disposition, revealed that the modified %absorbed *versus* time curves are of bilinear type with an ascending limb intersecting the horizontal line at F.A.T. A computer-based methodology is described for the estimation of F.A.T. from experimental data. More than one linear ascending limb is found when more than one absorption phase is operating. Experimental data were analyzed and the estimates for F.A.T were found to be similar to those derived from nonlinear regression analysis using PBFTPK models.

**Conclusion** These results place an end to the routinely reported exponential %absorbed *versus* time curves prevailing in biopharmaceutics-pharmacokinetics since their inception in the'60 s. These findings point to the use of the F.A.T. concept in drug absorption research and regulatory guidelines such as deconvolution techniques for the assessment of drug input rate, stochastic mean absorption time calculations, population analyses, *in vitro in vivo* correlations and bioequivalence guidelines.

**Keywords** biopharmaceutics · finite absorption time · oral drugs · pharmacokinetics

## Introduction

In the early days of Biopharmaceutics-Pharmacokinetics, Wagner and Nelson introduced the fundamental concept of percent absorbed *versus* time plots [1, 2]. These plots have been used extensively since they summarize the rate of drug input into the general circulation for one-compartment model drugs in a concise manner. Besides, these plots have been used for the estimation of the first-order absorption rate constant, $k_a$. Few years later, Loo and Riegelman [3, 4] extended the construction of percent absorbed *versus* time plots to two-compartment model drugs. Both techniques [1–3] can be applied under any type of absorption kinetics [1–4]. However, the infinite drug absorption notion associated with first order absorption kinetics, assumed in the subsequent analysis, has been criticized as an unphysical hypothesis [5]. In the late 1970s, numerical deconvolution emerged as an alternative method of calculating drug input rates [6]. Since then, multiple applications of deconvolution techniques have been published in the literature [7].

The construction of percent absorbed *versus* time plots is a basic element of *in vitro-in vivo* correlations (*IVIVC*), which play an important role in the production and approval of drug products and formulations. This is so since a successful *IVIVC* for a set of formulations implies that *in vitro*

✉ Panos Macheras
macheras@pharm.uoa.gr

Athanasios A. Tsekouras
thanost@chem.uoa.gr

[1] Faculty of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece

[2] PharmaInformatics Unit, ATHENA Research Center, Athens, Greece

[3] Department of Chemistry, National and Kapodistrian University of Athens, Athens, Greece

dissolution tests can be used in place of further bioequivalence studies in the production or modification of different formulations [8]. In some cases, the so called "level A" *IVIVC* can be achieved when the % absorbed profile, in essence, represents the *in vivo* "dissolution profile", which coincides point by point with the *in vitro* dissolution data [9].

All above techniques have been used over the past 60 years in the field of pharmacokinetics [5]. However, it has been recently demonstrated that the concept of infinite absorption time, associated with the absorption rate constant, which drives drug's absorption rate, is not physiologically sound [5, 10–13]. The first-order absorption notion seriously affects all calculations applied to the construction of the percent absorbed *versus* time plots [1–5], which are essential elements for the *IVIVC* plots. The recent analysis of oral drug absorption based on the finite absorption time (F.A.T.) concept [10] and the relevant physiologically based finite time pharmacokinetic (PBFTPK) models developed provided meaningful and reliable estimates for the duration of drug absorption as well as for the corresponding drug input rate(s) [10–16]. The differential equations of the PBFTPK models rely on the principles of passive drug absorption under sink conditions lasting for a F.A.T. [10, 13, 14]; for each one of the four Biopharmaceutics Classification System (BCS) drug classes [17–19] the input rate is linked with the limiting property, i.e., solubility or permeability [10, 16]. More than one input rate is observed when complex drug absorption is encountered [11, 13].

These advances prompted us to reconsider the percent absorbed *versus* time plots in terms of the F.A.T. concept. Accordingly, we modified the classical approaches [1–3] considering the termination of drug absorption, $\tau$, which corresponds to F.A.T. We further applied the modified approach to simulated and experimental literature data. We developed a computer-based technique for the estimation of the value of a F.A.T. from experimental data for the proper construction of the percent absorbed *versus* time plots.

## Theory

### The PBFTPK Models

According to the F.A.T. concept [10] oral drug absorption takes place under sink conditions with one or more sequential constant input rates [10–14] lasting for a total time $\tau$ less than 5 h for intestinal drug absorption or less than 30 h when colon drug absorption is also operating. The F.A.T. is the total time duration of the absorption phases, $\tau$. The value of $\tau$ is either equal to $t_{max}$, which is the time corresponding to maximum blood concentration of drug $C_{max}$, or is higher than $t_{max}$, i.e., it is observed at the descending limb of blood concentration–time curve [14]. For immediate release formulations the value of $\tau$ is dependent on the biopharmaceutical properties (solubility, permeability) of drug as well as its elimination characteristics [12]. Accordingly, the PBFTPK models developed [13] have one or more sequential constant drug input rate(s) of not necessarily equal duration.

### Percent Absorbed *versus* Time Plots

The construction of the fraction of dose absorbed *versus* time curve is a pivotal element for the development of *IVIVC*. All methodologies employed, Wagner-Nelson [1, 2], Loo-Riegelman [3] and deconvolution techniques, e.g., [6, 7], rely on the use of the area $[AUC]_0^\infty$ for normalization purposes. For example, Eq. 1 gives the fraction of dose absorbed up to time $t$, $[AUC]_0^t$ in accord with the Wagner-Nelson method [1, 2].

$$\frac{A_t}{A_\infty} = \frac{C_t + k_{el}\int_0^t Cdt}{k_{el}\int_0^\infty Cdt} = \frac{C_t + k_{el}[AUC]_0^t}{k_{el}[AUC]_0^\infty} \tag{1}$$

where $A_t$ is the amount absorbed up to time $t$, $A_\infty$ is the amount absorbed at infinite time, and $k_{el}$ is the elimination rate constant.

Under the F.A.T. notion, absorption terminates at time $\tau$ and therefore Eq. 1 should be changed to Eq. 2 whereas $[AUC]_0^\infty$ is replaced by $[AUC]_0^\tau$, $A_\infty$ is replaced by $A_\tau$ to which it is equal (see appendix in Supplementary Information for details) and the blood drug concentration at the end of the absorption process, $C_\tau$ is added in the denominator for mass balance purposes

$$\frac{A_t}{A_\tau} = \frac{C_t + k_{el}\int_0^t Cdt}{C_\tau + k_{el}\int_0^\tau Cdt} = \frac{C_t + k_{el}[AUC]_0^t}{C_\tau + k_{el}[AUC]_0^\tau} \text{ for } t \le \tau \tag{2}$$

As shown in the Appendix, for a single input stage we get,

$$\begin{aligned} \frac{A_t}{A_\tau} &= \frac{t}{\tau} \text{ for } t \le \tau \\ \frac{A_t}{A_\tau} &= 1 \text{ for } t > \tau \end{aligned} \tag{3}$$

For two compartment model drugs, Loo and Riegelman [3, 4] proposed Eq. 4 for the fraction of dose absorbed up to time $t$, which is symbolized with $t_n$ as originally suggested [3, 4],

$$\frac{A_{t_n}}{A_\infty} = \frac{C_{1_{t_n}} + C_{2_{t_n}} + k_{el}\int_0^{t_n} C_1 dt}{k_{el}\int_0^\infty C_1 dt} = \frac{C_{1_{t_n}} + C_{2_{t_n}} + k_{el}[AUC]_0^{t_n}}{k_{el}[AUC]_0^\infty} \tag{4}$$

where $C_{1_{t_n}}$, is the drug concentration in the central compartment and $C_{2_{t_n}}$ is the concentration of drug in the peripheral compartment. The latter is calculated [3, 4] from Eq. 5

$$C_{2_{t_n}} = \frac{k_{12}}{k_{21}} C_{1_{t_{n-1}}} \left[1 - e^{-k_{21}\Delta t}\right] + \frac{k_{12}\Delta C_1 \Delta t}{2} + C_{2_{t_{n-1}}} e^{-k_{21}\Delta t} \tag{5}$$

where $t_n$ is the sampling time of sample $n$, $t_{n-1}$ is the sampling time of the sample preceding sample $n$, $C_{1_{t_{n-1}}}$ is the concentration of drug at central compartment for sample $n$-1, $k_{12}$, $k_{21}$ are the first-order micro-constants for the drug transfer from the central compartment to peripheral compartment and vice versa, respectively; $\Delta C_1$ is equal to $C_{1_{t_n}} - C_{1_{t_{n-1}}}$ and $\Delta t$ is equal to $t_n$- $t_{n-1}$. Since the concentration of drug in the peripheral compartment is zero at the start of the study, when evaluating the concentration at $t_1$ it is appropriate to set the last term in Eq. 5 equal to zero. At each subsequent time, $t_n$ the drug concentration in the peripheral compartment must be solved by a method of successive substitution of the peripheral concentration from the previous time interval.

Equation 4 can now be written in accord with the F.A.T. concept,

$$\frac{A_{t_n}}{A_\tau} = \frac{C_{1_{t_n}} + C_{2_{t_n}} + k_{el}[AUC]_0^{t_n}}{C_{1\tau} + C_{2\tau} + k_{el}[AUC]_0^{\tau}} \text{ for } t \le \tau \tag{6}$$

where $C_{2_{t_n}}$ is given by Eq. 5.

Again, Eq. 3 holds for a single input stage and its proof is given in the Appendix. As is the case for the one-compartment model, beyond time $\tau$, this quantity is equal to unity.

## Methods

We simulated curves showing the amount of drug absorbed as a function of time based on the F.A.T. concept and we converted published pharmacokinetic data to determine amount of drug absorbed and establish the duration of absorption. Simulated errorless data using the equations listed in the Appendix for classical and PBFTPK models with different input rate scenarios were generated and the percent absorbed *versus* time plots were constructed using Eqs. 1, 2, 4 and 6.

Regarding the experimental data, we first analyzed them mathematically to determine pertinent parameters for classical and PBFTPK one- and two-compartment models including one or two input stages [13]. Using the resulting elimination rate constants required for the application of Eqs. 1 and 4 for the classical approach and Eqs. 2 and 6 for the F.A.T. approach, we calculated in all cases the amounts of drug absorbed as a function of time. The amounts of drug absorbed which appear in the numerators and denominators of Eqs. 1, 2, 4 and 6, are only known within an unknown scaling factor which is the volume of distribution, $V_d$, or the volume of the central compartment, $V_c$, depending on whether the drug is described by a one- or a two-compartment model, respectively. Hence, numerators and denominators, as written in Eqs. 1, 2, 4 and 6, are, strictly speaking, concentrations, although they are proportional to the amount of drug absorbed. We will use the term "apparent absorbed concentration" (AAC) to refer to the amount of drug absorbed, $A$, divided by the unknown volume of distribution. A fitting procedure was then followed for all curves of the apparent absorbed concentrations to determine the duration of F.A.T.

## Results

### Simulated Data

The percent absorbed *versus* time plots for the simulated errorless data are presented in Fig. 1. The results of Fig. 1c and d correspond to drugs following one- or two-compartment model disposition with the termination of drug absorption at $t_{max}$, i.e., $t_{max} = \tau$ [14]. Figure 1e and f show the percent absorbed *versus* time plots for data exhibiting two input stages and termination of drug absorption beyond $t_{max}$, i.e., $t_{max} < \tau$ [14].

In all simulated data sets (Fig. 1) generated from the classical equations (A1, A4, A9, A12) with first-order absorption, the exponential increase of percent absorbed *versus* time curve reaching asymptotically the plateau value, 1, is observed. It should be noted that most of percent absorbed *versus* time plots reported in the literature for the last fifty years or so exhibit an exponential increase since first-order absorption for one or two compartment model drugs is routinely used. On the contrary, the percent absorbed *versus* time plots adhering to data generated from the PBFTPK models, exhibit either a single limb or two limbs when one (A12, A18, A19, A22; A39, A40, A45, A46, A47, A50) or two input rates (A23, A28, A29, A33, A34, A38; A51, A52, A57, A58, A59, A60, A61, A62, A63) are operating, respectively. There is always a specific time point, $\tau$, corresponding to the intersection of the two linear segments, that of the ascending limb and the horizontal line at the plateau.

The simulated data are in full accord with the mathematical analysis of the PBFTPK models presented in the Appendix. Most importantly it is shown that the value of the ratio of percent absorbed, $A_t/A_\tau$ is equal to one when $t \ge \tau$ in all PBFTPK models examined (Eqs. A22, A38, A50, A63). Besides, there is a linear increase of the ratio $A_t/A_\tau$ as a function of time for $t \le \tau$ (A18, A45) or $t \le \tau_1$ (A28, A57) or $t \le \tau_1 + \tau_2$ (A33, A60). Both characteristics, namely, the linear increase of $A_t/A_\tau$ during the successive absorption stages followed by a plateau value equal to one are clearly depicted in Fig. 1c,d,e,f. Based on these

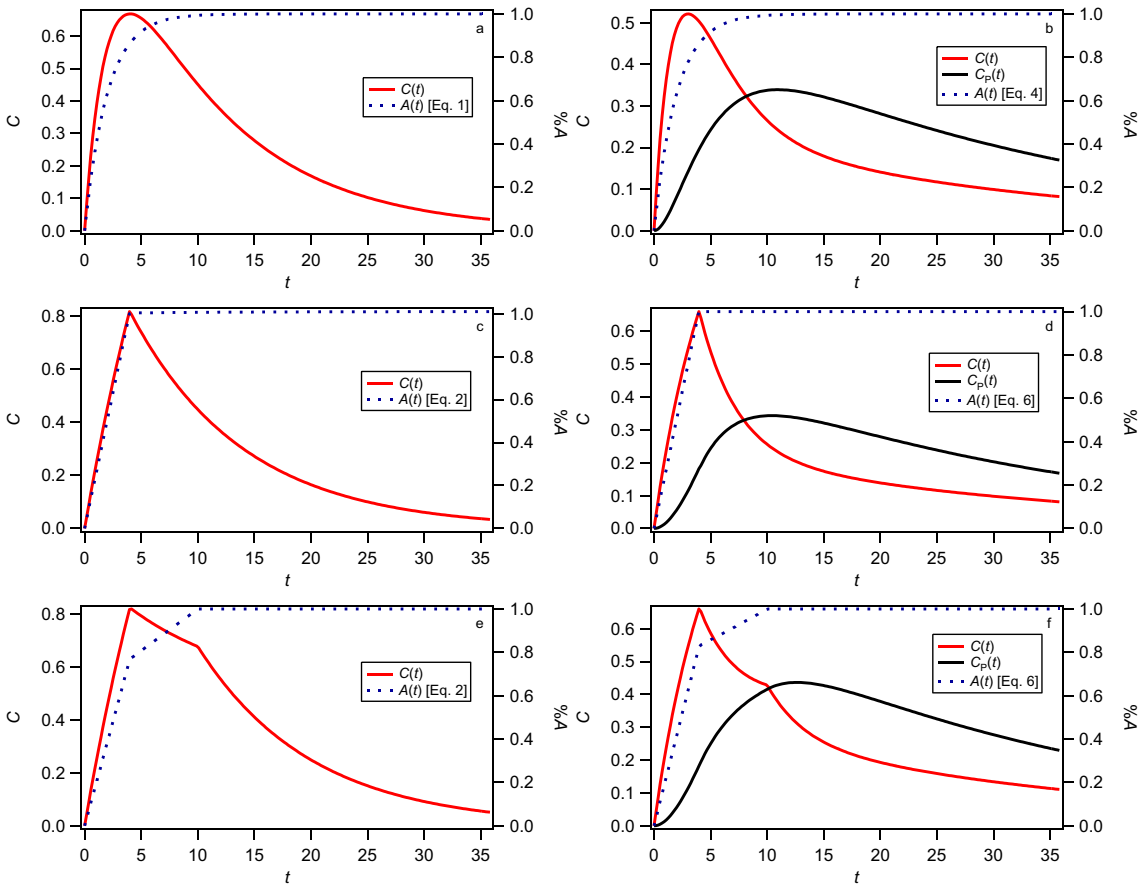


**Fig. 1** Simulated oral pharmacokinetic data showing drug concentration, $C$, in the blood (red line) and in the peripheral compartment (where pertinent, black line) and percent drug absorbed (dashed blue line) using one- (a, c, e) and two-compartment (b, d, f) models based on first-order infinite time absorption (**a**, **b**) and finite time, zero-order absorption (**c**-**f**). In c and d, $\tau_{max}=\tau$, whereas in e and f, $\tau_{max}<\tau_1+\tau_2$. Parameters used for the equations quoted in the Appendix: a: $FD/V_d=1$, $k_{el}=0.1$, $k_a=0.5$; b: $FD/V_c=1$, $k_{10}=0.1$, $k_{21}=0.1$, $k_{12}=0.14$; c: $FD/V_d=1$, $k_{el}=0.1$, $\tau=4$; d: $FD/V_d=1$, $k_{10}=0.1$, $k_{21}=0.1$, $k_{12}=0.14$, $\tau=4$; e: $F_1D/V_d=1$, $F_2D/V_d=0.3$, $k_{el}=0.1$, $k_{21}=0.1$, $k_{12}=0.14$, $\tau_1=4$, $\tau_2=6$; f: $F_1D/V_c=1$, $F_2D/V_c=0.3$, $k_{10}=0.1$, $k_{21}=0.1$, $k_{12}=0.14$, $\tau_1=4$, $\tau_2=6$.

observations, the percent absorbed *versus* time curve as a function of time is bilinear, when an input stage is operating, or multi-linear, when more than one input phase is observed, Fig. 1.

The generated curves from PBFTPK models with two compartment disposition have the same shape. In all cases, the horizontal line (100% absorbed) always intersects with the ascending final linear segment at time $\tau$, which denotes the end of the absorption processes. In view of all above, we developed a computer-based approach for the estimation of $\tau$ from experimental data, by calculating $A_t/V_d$ as a function of time using combinations of experimental data points from the two segments of the bilinear plot. The best estimate for $\tau$ is determined by the sum of squares of deviations from the two lines, i.e., the partitioning of the data is scanned in search of the minimum value of this sum. This approach is described below and is applied to a series of experimental data delineated below.

## Experimental Data

The detailed analysis of experimental data is presented in Figs. 2, 3 using cyclosporine data [13] to provide details for the procedures followed. In Fig. 2, the continuous line connects the points that correspond to the apparent absorbed concentration (numerator of Eqs. 1 and 4 divided by the volume of distribution) and the dashed lines show the absorption profiles based on F.A.T. (Eq. 3). The ascending part of the curve determines the sloping line, and the average of remaining data points defines the level of the horizontal line. Their intersection determines the end of the absorption stage, i.e., $\tau$. This calculation depends on the choice of which points belong to the first segment and which ones to the second one. The intermediate point is allowed to be part of both segments. The best choice is determined by the sum of squares of deviations from the two lines, $\chi^2$. The partitioning of the data is scanned in search of the minimum value of this

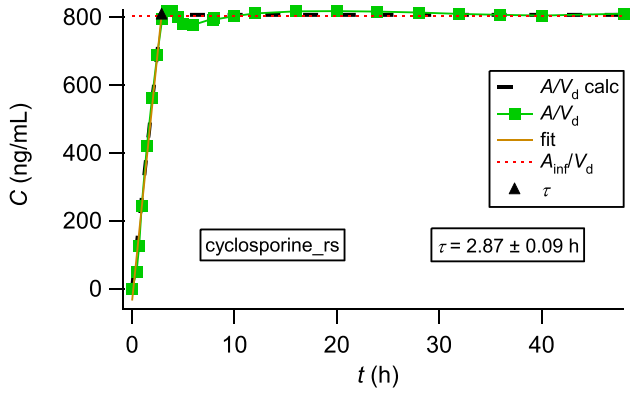


**Fig. 2** Percent absorbed (expressed as apparent absorbed concentration) *versus* time plot (green squares and solid line) for cyclosporine, reference formulation, fasted subjects [13]. Optimum partitioning of the data in two segments was found at $i=8$ as seen in Fig. 3. The intersection of the two straight segments corresponds to the termination of cyclosporine absorption. For this example, the optimum estimate for $\tau$ is $2.87 \pm 0.09$ h (see Fig. 3). The black triangle denotes the termination of drug absorption. The black dashed line is the simulation based on the model (Eq. 6). The brown solid line is the fit of Eq. 6 to the ascending segment data; the red dotted lines are the average level of the plateau values.

sum. The values of $\chi^2$ for this data set are presented in Fig. 3 and Table S1 as a function of the index of the turning point. Shown is also the corresponding value of the absorption time, $\tau$, and its associated uncertainty, $\sigma_\tau$. The minimum in $\chi^2$ coincides with the $\tau$ value with lowest uncertainty. The values for $\tau$, slope and plateau level at the least value of $\chi^2$ are taken as the optimum values.

The analysis of experimental data [13] is presented in Figs. S1-S5 in the Supplementary Information. The classical and PBFTPK one- and two-compartment models fittings to experimental data are also presented in Figs. S1-S5 for comparative purposes; the fitted estimate of the elimination rate

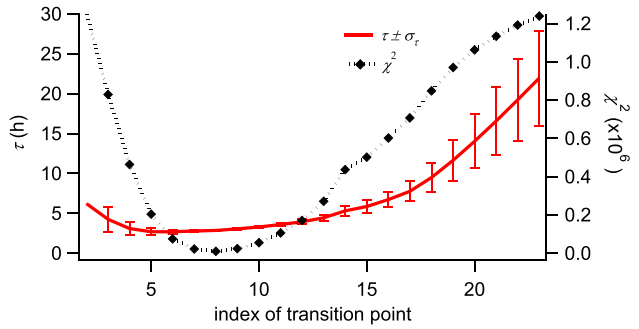


**Fig. 3** Investigation of the effect of partitioning of cyclosporine apparent absorbed concentration in rising and plateau segments. Shown are the values (and associated errors) for $\tau$, (red solid line) as well as $\chi^2$ values (black dotted line) as a function of index of point where the two segments meet. The minimum value for $\chi^2$ determines the optimum selection of the transition point. The same data are listed in Table S1.

constant required for the application of Eqs. 1 and 4 for the classical approach and Eqs. 2 and 6 for the F.A.T. approach was used for the construction of percent absorbed (expressed as apparent absorbed concentration) *versus* time plots. In all these plots the continuous lines correspond to the effective concentration (numerator of Eqs. 1 and 4 divided by the volume of distribution) and the dashed lines show the absorption profiles based on F.A.T. (Eq. 3).

Figure S1 shows the percent absorbed *versus* time plot of paracetamol generated from the fitting results described in [13]. The absorption of paracetamol proceeds very rapidly and the estimate for $\tau$ is very close to 0.5 h regardless the estimation approach utilized. According to common scientific belief, the absorption of oral paracetamol occurs primarily along the small intestine by passive diffusion and the rate-limiting step is the rate of gastric emptying into the intestines. However, a plethora of data shows that the absorption of paracetamol is very rapid, e.g., a mean $t_{max}$ of $0.875 \pm 0.44$ h is reported [20] and a major review [21] quotes that it is rapidly absorbed from the gastrointestinal tract and its systemic bioavailability ranges from 70 to 90%. This observation for paracetamol most likely means that all estimates for $\tau$, quoted in Fig. S1, do not simply signify the termination of its absorption, but the completion of the absorption process, i.e., the entire dose has been absorbed and no more drug is available for absorption. In addition, one must recall here hundreds of millions of people who consume paracetamol or its combination during almost any type of symptoms, who experience its rapid pharmacological action and therefore its rapid absorption.

Figure S2 shows the results of ibuprofen absorption [13]. The two stages of ibuprofen absorption, observed in the PBFTPK fitting (Fig. S2c), correspond to the two limbs of the ascending lines of the percent absorbed *versus* time plots (Fig. S2d) resulting in statistically equivalent estimates for $\tau$, $2.3 \pm 0.2$ h and $2.28 \pm 0.13$ h. This means that the termination of ibuprofen absorption takes place at 2.3 h in the small intestines. On the contrary, the estimate for $\tau$ derived from the classical method (Fig. S2b) is much shorter ~ 1.5 h.

Figure S3 shows the results of almotriptan absorption [13]. The estimates for $\tau$ derived from the classical methods (Fig. S3a-d) differ remarkably, $2.2 \pm 0.2$ h and $4.5 \pm 0.8$ h. This difference is associated with the method of estimation of the elimination rate constant, i.e., semi-logarithmic approach using all elimination phase data (Fig. S3a) and best nonlinear fit estimate of the classical one-compartment model with equal rate constants (Fig. S3c). The best estimate for $\tau$, $3.1 \pm 0.2$ h is derived when two ascending linear segments are utilized (Fig. S3f) in accord with the best PBFTPK modeling fitting (Fig. S3e). These results substantiate the view that almotriptan absorption is terminated in the small intestines.

Figure S4 presents the analysis of cyclosporine data derived from the bioequivalence study under fasted and fed conditions reported in [13]. In all cases, the estimates for $\tau$ based on the classical two compartment model fits are much higher with larger uncertainties compared to the estimates for $\tau$ based on the PBFTPK modeling and the F.A.T. concept (visually compare the estimates in each one of the right panels *vis a vis* its succeeding panel in Fig. S4). Similarly, visual inspection of $\tau$ estimates in Fig. S4 reveals the more rapid absorption of cyclosporine from the test formulation than the reference formulation. The relevant $\tau$ estimates can be seen in Table I.

Figure S5 shows the results of niraparib absorption [13]. Although the $\tau$ estimates using the classical approach and the F.A.T. based approach are statistically equivalent, $3.3 \pm 0.2$ h and $3.3 \pm 0.5$ h one can mention the higher uncertainty of the estimate derived from the classical approach. This is due to the poor fitting of the classical two-compartment model with classical first-order absorption (Fig. S5a); this poor fitting is also reflected in the curvature observed in the "horizontal" segment of the percent absorbed *versus* time plot (Fig. S5b). However, this example shows that the application of the F.A.T. concept in a drug with extreme prolonged disposition profile like niraparib, can provide a reliable estimate for $\tau$ using a classical two-compartment model with first-order absorption.

Table I shows an overview of the $\tau$ estimates derived in this work *vis a vis* the estimates obtained using nonlinear regression analysis on PBFTPK models [13]. Statistically equivalent estimates are determined with the two approaches. These results substantiate the view that the F.A.T. concept is physiologically and physically sound and its valid estimation can be accomplished with the technique developed herein.

**Table I** Estimates for $\tau$ and their Uncertainties Derived in [13] using Non-linear Regression Analysis based on PBFTPK Modeling along with the Estimates Derived in the Present Study based on the Absorbed Amount

| | PBFTPK | | Absorbed amount | | 1 or 2 |
|---|---|---|---|---|---|
| Data set | $\tau$ (h) | $\sigma_\tau$ (h) | $\tau$ (h) | $\sigma_\tau$ (h) | Compartments |
| Paracetamol | 28 min | 2 min | 29 min | 3 min | 1 |
| Ibuprofen | 2.3 | 0.2 | 2.2 | 0.14 | 1 |
| Almotriptan | 3.15 | 0.3 | 3.1 | 0.2 | 1 |
| Cyclosporine, ref, fasted | 2.9 | 0.1 | 2.87 | 0.1 | 2 |
| Cyclosporine, ref, fed | 4.7 | 0.1 | 5.2 | 0.3 | 2 |
| Cyclosporine, test, fasted | 1.56 | 0.15 | 1.5 | 0.1 | 2 |
| Cyclosporine, test, fed | 1.73 | 0.13 | 1.63 | 0.13 | 2 |
| Niraparib | 3.5 | 0.1 | 3.3 | 0.2 | 2 |

## Discussion

All above results associated with the modified, in terms of the F.A.T. concept, Wagner-Nelson and Loo-Riegelman equations have a broader interest beyond the percent absorbed *versus* time plots. In fact, these developments open a new horizon for applications of the F.A.T. concept in the whole spectrum of biopharmaceutic-pharmacokinetic and pharmacometric studies as well as in regulatory science of drug absorption studies. In this vein, we anticipate important implications or changes in the following topics dealing with oral drug absorption.

### Drug Dissolution

A plausible, direct consequence of the F.A.T. concept is the consideration of the *in vivo* drug dissolution in terms of physiological time constraints. In fact, *in vivo* drug dissolution runs for a finite dissolution time (F.D.T.), $\tau_d$, which is equal or shorter than F.A.T., $\tau_d \leq \tau$. Intuitively, for BCS Class II drugs $\tau_d = \tau$, while for Class I and III drugs $\tau_d < \tau$. Accordingly, modified in terms of finite dissolution time functions must be applied in future work for the analysis of *in vitro* and *in vivo* dissolution data. Such an approach has been developed for the Noyes Whitney and Weibull functions used routinely in drug dissolution [22].

### *In vitro in vivo* Correlations (IVIVC)

The major take home message of the present work is that the calculations of the percent absorbed drug using Eqs. 2 and 6 are physically and physiologically correct. Therefore, their use in the construction of *IVIVC* is scientifically sound. In parallel, physiologically based time constraints for the % of drug dissolved will be gradually applied as delineated in the previous paragraph. This will lead to better *IVIVC* improving the predictability of published [23] and most importantly the failed not published *IVIVC* [24]; such a development can result in a potential amendment of the relevant guidelines, e.g., [9]. In the last twenty years or so, the attention has been focused on biorelevant dissolution media [19] as a rather utopic panacea for the prediction of the *in vivo* drug dissolution ignoring [25] the physiologically sound time constraints, i.e., F.A.T. and F.D.T. concepts. The current work points to a re-analysis of dissolution studies in classical and biorelevant media under the prism of F.A.T. and F.D.T. concepts using functions of finite time [22].

### Development of Generics

The advances quoted in the two previous paragraphs can also be applied to the development of generics. Analysis of the *in vitro* and *in vivo* data of the reference formulation as delineated above, can provide the real picture of *IVIVC*, if any, for the reference formulation. This result can be further used as a guide for the development of generic formulation.

### Biowaivers

According to a biowaivers guidelines [26], "a BCS-based biowaiver for BCS Class I drug substances should display either very rapid ($\geq 85\%$ for the mean percent dissolved in $\leq 15$ min) *in vitro* dissolution characteristics, or rapid ($\geq 85\%$ for the mean percent dissolved in $\leq 30$ min)". This implies that the F.A.T. and F.D.T. concepts lie in the heart of the guideline since the absorption of a biowaiver drug is terminated or, better, completed in a very short and specific ($\leq 15$ min or $\leq 30$ min) period. This opens a new field of pharmacokinetic calculations for the estimation of absolute bioavailability of biowaivers from oral data exclusively using the F.A.T. concept as exemplified in [12] for theophylline.

### Bioequivalence Studies

The termination or completion of absorption at time $\tau$ has a great impact on the use of the parameters $C_{max}$ and $AUC$ for the assessment of bioequivalence studies [12, 15, 17]. In addition, a methodology for the revision of the assessment of bioequivalence has been presented recently [27]. However, the present work points out specifically on the validity of important aspects of the bioequivalence guidelines associated with the sampling scheme and the duration of sampling. Thus, the two recommendations of the current bioequivalence guidelines [28, 29], namely, i) "The sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the extent of exposure which is achieved if $[AUC]_0^t$ covers at least 80% of $[AUC]_0^\infty$" and ii) the specific time limit of 72 h, for the calculation of total $AUC$, i.e., "$AUC$ truncated at 72 h ($[AUC]_0^{72}$) may be used as an alternative to $[AUC]_0^t$ for comparison of extent of exposure as the absorption phase has been covered by 72 h for immediate release formulations", should be re-considered in view of the F.A.T concept and the physiological time limit for gastrointestinal drug absorption of 30 h [10].

### Mean Time Estimates

Stochastic approaches based on mean absorption and mean residence time estimates have been and are still used in pharmacokinetics for the description of time course of drug in the body [30, 31]. In most cases, drug absorption is based on the first-order hypothesis and the mean absorption time corresponds to the reciprocal of the absorption rate constant. Our work shows that zero-order considerations of single or multiple successive absorption phases should be used instead.

### Physiologically Based Pharmacokinetic (PBPK) Models and Pharmacometrics

The PBPK models have been used for the last twenty years or so for the analysis and modeling of absorption phenomena and the prediction of pharmacokinetic profiles [32, 33] based on drug's physicochemical and biopharmaceutical properties. In essence, they are "bottom up" models, while PBFTPK models, which rely on the F.A.T. concept, are "top down" models. Their obvious complementarity will contribute to a better understanding of the drug absorption phenomena. Recent relevant work paved the way [11, 15] towards this end. Pharmacometric studies utilize as structural models either one or two compartment models assuming first-order absorption; therefore, the use of PBFTPK models will enhance the analytical power of population analyses dealing with drug absorption phenomena. A relevant application was recently published [11].

### Drug Structure-oral Absorption Relationships

The slope of the initial linear increase of percent absorbed *versus* time plots, e.g., Fig. 2, is a measure of the drug's input rate. The magnitude of the slope and the duration of input, $\tau$ can be coupled with drug's structural properties, e.g., molecular descriptors, or physicochemical properties, e.g., lipophilicity or biopharmaceutical properties, e.g., solubility, permeability, for the development of relationships. This type of work can be carried out using big data analysis tools to disrupt-revolutionize the traditional structure-absorption models.

## Conclusion

The results of the present study are an additional piece of evidence against the routinely reported [34] infinite time of drug absorption used in biopharmaceutics-pharmacokinetics since 1953. This disruptive work completely revises the construction of the percent absorbed *versus* time plots using the physiologically sound F.A.T. concept. A methodology for the reliable estimation of F.A.T. from experimental data is described. Numerous applications of the use of F.A.T. concept in biopharmaceutics, pharmacokinetics, PBPK, and pharmacometrics are anticipated.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11095-023-03578-x.

**Acknowledgements** The authors are indebted to Professor Leslie Benet for his advice on an advanced draft of the manuscript. We also thank an anonymous reviewer for his constructive critique. Dedicated to the memory of the hero pilots Christos Moulas, 34, and Periclis Stefanidis, 27, both of the Greek Hellenic Air Force who died fighting devastating wildfires in Greece on July 25, 2023.

**Funding** This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**Data Availability** All data used in this study have been published previously as indicated for each data set analyzed.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

## References

1. Wagner JG, Nelson E. Percent absorbed time plots derived from blood level and/or urinary excretion data. J Pharm Sci. 1963;52:610–1. https://doi.org/10.1002/jps.2600520629.
2. Wagner JG, Nelson E. The kinetic analysis of blood levels and urinary excretion in the absorptive phase after single doses of drug. J Pharm Sci. 1964;53:1392–403. https://doi.org/10.1002/jps.2600531126.
3. Loo JCK, Riegelman S. New method for calculating the intrinsic absorption rate of drugs. J Pharm Sci. 1968;57:918–28. https://doi.org/10.1002/jps.2600570602.
4. Wagner JG. Application of the Loo-Riegelman absorption method. J Pharmacokinet Biopharm. 1975Feb;3(1):51–67. https://doi.org/10.1007/BF01066595.
5. Macheras P. On an unphysical hypothesis of Bateman equation and its implications for pharmacokinetics. Pharm Res. 2019;36:94. https://doi.org/10.1007/s11095-019-2633-4.
6. Cutler DJ. Numerical deconvolution by least squares: use of prescribed input functions. J Pharmacokinet Biopharm. 1978;6(3):227–41. https://doi.org/10.1007/BF01312264.
7. Margolskee A, Darwich AS, Galetin A, Rostami-Hodjegan A, Aarons L. Deconvolution and IVIVC: Exploring the Role of Rate-Limiting Conditions. AAPS J. 2016Mar;18(2):321–32. https://doi.org/10.1208/s12248-015-9849-y.
8. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry: SUPAC-MR: Modified Release Solid Oral Dosage Forms. 1997. http://www.fda.gov/downloads/Drugs/Guidances/UCM070640.pdf. Accessed Feb 20 2023
9. Uppoor VR, *et al*. FDA Guidance to Industry Extended Release Oral Dosage Forms: Development, Evaluation, And Application Of In Vitro/In Vivo Correlations. Dissolution Technol. 1997;4(4):23–32. https://doi.org/10.14227/DT040497P23.
10. Macheras P, Chryssafidis P. Revising pharmacokinetics of oral drug absorption: I models based on biopharmaceutical/physiological and finite absorption time concepts. Pharm Res. 2020;37:187. https://doi.org/10.1007/s11095-020-02894-w.ErratumPharmRes2020;37:206.
11. Macheras P, Tsekouras AA. The Finite Absorption Time (FAT) concept en route to PBPK modeling and pharmacometrics. J Pharmacokinet Pharmacodyn. 2023;50:5–10. https://doi.org/10.1007/s10928-022-09832-w.
12. Chryssafidis P, Tsekouras AA, Macheras P. Revising pharmacokinetics of oral drug absorption: II bioavailability bioequivalence considerations. Pharm Res. 2021;38:1345–56. https://doi.org/10.1007/s11095-021-03078-w.
13. Chryssafidis P, Tsekouras AA, Macheras P. Re-writing oral pharmacokinetics using physiologically based finite time pharmacokinetic (PBFTPK) models. Pharm Res. 2022;39:691–701. https://doi.org/10.1007/s11095-022-03230-0.
14. Tsekouras AA, Macheras P. Columbus' egg: Oral drugs are absorbed in finite time. Eur J Pharm Sci. 2022;176:106265. https://doi.org/10.1016/j.ejps.2022.106265.
15. Wu D, Tsekouras AA, Macheras P, Kesisoglou F. Physiologically based pharmacokinetic models under the prism of the finite absorption time concept. Pharm Res. 2023;40:419–29. https://doi.org/10.1007/s11095-022-03357-0.
16. Macheras P, Tsekouras AA. Revising Oral Pharmacokinetics, Bioavailability and Bioequivalence Based on the Finite Absorption Time Concept. Cham: Springer; 2023. https://doi.org/10.1007/978-3-031-20025-0.
17. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. Guidance for Industry U.S. Department of Health and Human Services. 2017.
18. European Medicines Agency. Committee for medicinal products for human use (CHMP) Guideline on the investigation of bioequivalence. London, Jan. 2017.
19. Charalabidis A, Sfouni M, Bergström C, Macheras P. BCS and BDDCS: Beyond guidelines. Int J Pharm. 2019;566:264–81. https://doi.org/10.1016/j.ijpharm.2019.05.041.
20. Raffa RB, Pergolizzi JV Jr, Taylor R Jr, Decker JF, Patrick JT. Acetaminophen (paracetamol) oral absorption and clinical influences. Pain Pract. 2014Sep;14(7):668–77. https://doi.org/10.1111/papr.12130.
21. Forrest JA, Clements JA, Prescott LF. Clinical pharmacokinetics of paracetamol. Clin Pharmacokinet. 1982;7(2):93–107. https://doi.org/10.2165/00003088-198207020-00001.
22. Dokoumetzidis A, Papadopoulou V, Macheras P. Analysis of Dissolution Data Using Modified Versions of Noyes-Whitney Equation and the Weibull Function. Pharm Res. 2006;23:256–61. https://doi.org/10.1007/s11095-006-9093-3.
23. Dokoumetzidis A, Macheras P. IVIVC of controlled release formulations: physiological-dynamical reasons for their failure. J Control Release. 2008Jul 14;129(2):76–8. https://doi.org/10.1016/j.jconrel.2008.04.005.
24. Mircioiu C, Mircioiu I, Voicu V, Miron D. Dissolution-bioequivalence non-correlations. Basic Clin Pharmacol Toxicol. 2005Mar;96(3):262–4. https://doi.org/10.1111/j.1742-7843.2005.pto960324.x.
25. Cardot JM, Davit BM. In vitro-in vivo correlations: tricks and traps. AAPS J. 2012Sep;14(3):491–9. https://doi.org/10.1208/s12248-012-9359-0.
26. Bransford P, Cook J, Gupta M, Haertter S, He H, Ju R, Kanodia J, Lennernäs H, Lindley D, Polli JE, Wenning L, Wu Y. ICH M9 Guideline in Development on Biopharmaceutics Classification System-Based Biowaivers: An Industrial Perspective from the IQ Consortium. Mol Pharm. 2020Feb 3;17(2):361–72. https://doi.org/10.1021/acs.molpharmaceut.9b01062.
27. Alimpertis N, Tsekouras AA, Macheras P, Revising the assessment of bioequivalence in the light of finite absorption time (FAT) concept: The axitinib case. 30th PAGE meeting Ljubljana 28 June-1 July, 2022.

28. Food and Drug Administration, Code of Federal Regulations Title 21, 5 Subpart B - Procedures for Determining the Bioavailability or Bioequivalence of Drug Products. 1971. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=320.21. Accessed 27 Jul 2023
29. European Medicines Agency. Committee for medicinal products for human use (CHMP) guideline on the investigation of bioequivalence. London, Jan. 2010. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-investigation-bioequivalence-rev1_en.pdf
30. Yamaoka K, Nakagawa T, Uno T. Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm. 1978;6:547–58. https://doi.org/10.1007/BF01062109.
31. Benet LZ, Sodhi JK. The Uses and Advantages of Kirchhoff's Laws vs. Differential Equations in Pharmacology, Pharmacokinetics, and (Even) Chemistry. AAPS J. 2023;25(3):38. https://doi.org/10.1208/s12248-023-00801-w.
32. Sjögren E, Westergren J, Grant I, Hanisch G, Lindfors L, Lennernäs H, *et al.* In silico predictions of gastrointestinal drug absorption in pharmaceutical product development: application of the mechanistic absorption model GI-Sim. Eur J Pharm Sci. 2013;49(4):679–98. https://doi.org/10.1016/j.ejps.2013.05.019.
33. Sager JE, Yu J, Ragueneau-Majlessi I, Isoherranen N. Physiologically based pharmacokinetic (PBPK) modeling and simulation approaches: a systematic review of published models, applications, and model verification. Drug Metab Dispos. 2015;43(11):1823–37. https://doi.org/10.1124/dmd.115.065920.
34. Alimpertis N, Tsekouras AA, Macheras P. The estimates for the absorption rate constant in pharmacokinetics and pharmacometrics are wrong: A new era based on the finite absorption time concept rises, talk presented at PAGE 2023, A Coruña, Spain, 28–30 June 2023. https://www.page-meeting.org/default.asp?abstract=10610. Accessed 27 Jul 2023