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RESEARCH PAPER

Revising Pharmacokinetics of Oral Drug Absorption: I Models Based on Biopharmaceutical/Physiological and Finite Absorption Time Concepts

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ABSRACT

Purpose To demonstrate that oral drug absorption is terminated in finite time. To develop models based on biopharmaceutical/physiological and finite absorption time concepts.

Methods The models are based on i) the passive drug diffusion mechanism under the sink conditions principle ii) the rate limiting role of the drug's properties solubility and permeability and iii) the relevant restrictions associated with the gastrointestinal transit times of drug in the stomach, the small intestines and the colon. Two input functions of constant rate are considered for the absorption of drug from i) the stomach/small intestines with an upper limit of 5 h and ii) the colon with an upper limit of 30 h. Branched differential equations were written for the time course of drug in the body.

Results Simulations were performed using different scenarios, assuming a variety of drug properties and limited or non-existent absorption from the colon. Literature oral data of cephradine, ibuprofen, flurbiprofen and itraconazole were analyzed. For all drugs examined, nice fittings of the branched differential equations to the experimental data were observed.

Conclusions For all drugs the absorption process was terminated in the small intestine. The meaning of partial AUCs, Cmax, tmax are questioned. Applications of these models to IVIVC are anticipated.

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KEY WORDS oral drug absorption · finite absorption time · pharmacokinetics, BCS, BDDCS

ABBREVIATIONS

BCS	Biopharmaceutic classification system
BDDCS	Biopharmaceutic drug disposition classification System
GI	Gastrointestinal
IVIVC	*In vitro in vivo* correlations
PBFTPK	Physiologically based finite time pharmacokinetic
PBPK	Physiologically based pharmacokinetic

INTRODUCTION

The oral route is the most common for drug administration. Extensive work in this field of research revealed that two basic drug properties, namely, solubility and permeability of gastrointestinal membrane determine the extent of oral drug absorption [1, 2]. These scientific advances lead to the development of the biopharmaceutic classification system (BCS), the biopharmaceutic drug disposition classification system (BDDCS) and the publication of relevant regulatory guidelines, by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) [3, 4]. These guidelines formulate the scientific requirements for the performance or not of bioequivalence studies towards the approval of generics/drugs classified in four drug classes (I, II, III, IV). For example, a highly soluble, highly permeable drug (Class I) can get a biowaiver status for bioequivalence studies (3–5). This does not apply to Class II (low solubility, high permeability), and Class IV (low solubility, low permeability) drugs. For Class III (high solubility, low permeability) a biowaiver status can be assigned under certain conditions [3, 4]. Class I drugs exhibit extensive absorption (fraction of dose

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absorbed >0.90), while for Class II, III and IV drugs the fraction of dose absorbed is certainly lower than 0.90.

However, it is very well known, that the absorption of orally administered drugs is complex and depends not only on drug properties but also on physiological aspects of the gastrointestinal (GI) tract [6] such as (a) drug/formulation-dependent factors—drug physicochemical properties [e.g., aqueous solubility, permeability, molecular size, aggregation/ complexation, charge, pKa, H-bonding potential, hydrophobicity, and crystal lattice energy] and formulation composition (e.g., dosage form, absorption enhancers, and drug release) and (b) system dependent factors—physiological parameters (e.g., gastric emptying, intestinal motility, intestinal pH, site-dependent permeability, intestinal content composition, and disease state) and biochemical parameters (e.g., metabolism, efflux transporters, and active uptake transporters). Due to this complexity during the last fifteen years or so different modeling approaches have been proposed and software packages (GastroPlus® Software, n.d.; Simcyp® Simulator, n.d.; PK-Sim® Software, n.d.) have been developed for the analysis of oral drug absorption. These advances have resulted in the development of physiologically based pharmacokinetic (PBPK) modeling field [5, 7–10].

One characteristic of paramount importance for all modeling approaches is the duration of the absorption process, the so called mean intestinal transit time. In the most of the cases, the user/modeler can fix the value to a finite time period e.g. 199 min [7, 11, 12] The selection of finite time is crucial for the predictive purposes of the model/software. However, in hundreds and hundreds of pharmacokinetic, pharmacokinetic-pharmacodynamic and pharmacometric studies dealing with oral drug absorption, the rate of drug input is routinely estimated with the absorption rate constant. This parameter is the hallmark of first-order rate of drug absorption, which is associated with an infinite absorption time [13]. Its use started in 1953 when Dost introduced the term pharmacokinetics [14] by adopting the relevant Bateman equation [15, 16] quoted in all pharmacokinetic textbooks.

The current work focuses on the duration of oral drug absorption. To this end, a physiologically based minimal model coupled with the principles of the biopharmaceutic classification system was constructed. The pharmacokinetic analysis of four drugs, namely, cephradine, ibuprofen, flurbiprofen and itraconazole demonstrated that their oral absorption is terminated at finite time coinciding with t_{max}.

THEORY

History

In 1910 Henry Bateman [15] described, using Eq.1 the abundances and activities of the daughter-isotope in a decay chain of three isotopes i.e. mother, daughter, grand-daughter as a function of time, Fig. 1. Based on the similarity of the kinetic processes depicted in Fig. 1, Friedrich Hartmut Dost [14] used Eq.1 in 1953 and described the blood concentration C_b in the body at time t, assuming one-compartment model disposition with first-order absorption and elimination rates.

$$C_b(t) = \frac{FDk_a}{V_d(k_a - k_{el})}\left(e^{-k_{el}t} - e^{-k_a t}\right) \quad (1)$$

where, F is the bioavailable fraction of dose (D), V_d is the volume of distribution and k_a, k_{el} are the absorption and elimination first-order rate constants, respectively. In physics, thousands of experimental observations have shown that the first-order decay of isotopes is undoubtedly true. Similarly, the prevailing first-order character of the elimination rate of drugs has been verified in numerous pharmacokinetic studies. On the contrary, the infinite time of drug absorption is not physiologically sound since drugs are not absorbed beyond their absorptive sites in the GI tract. In fact, oral drug absorption takes place in a certain period of time in accordance with the biopharmaceutical properties of the drug as well as the physiological gastric, intestinal and colon transit times reported in the literature [17].

Biopharmaceutical-Pharmacokinetic Considerations

Basically, drugs pass through the gastrointestinal membranes by passive diffusion. Fick's laws of diffusion describe the flux of solutes (drugs) undergoing classical diffusion. The simplest system to consider is a solution of a drug with two regions of different concentrations, C_{GI} at the absorption site of the gastrointestinal lumen and blood concentration, C_b of a boundary (GI membrane) separating the two regions. The driving force for drug transfer is the concentration gradient between the concentrations of the drug molecules in the two regions. Thus, the rate of penetration can be written [18]:

$$\textbf{\textit{Rate of Penetration}} = P\cdot(SA)\cdot(C_{GI} - C_b) \quad (2)$$

where P is the permeability of drug expressed in velocity units (length/time) and SA is the surface area of the membrane in $(\text{length})^2$ units. The sheer size of the body, by diluting absorbed drug, tends to maintain sink conditions, in which C_b is much smaller than C_{GI}, therefore,

$$\textbf{\textit{Rate of Penetration}} = P\cdot(SA)\cdot(C_{GI}) \quad (3)$$

Eq.3 can be written in terms of drug amount, A_{GI} assuming that the volume of fluid at the absorption site V_{GI} remains relatively constant,

$$\textbf{\textit{Rate of Penetration}} = P\cdot(SA)\cdot\frac{A_{GI}}{V_{GI}} = k_a A_{GI} \quad (4)$$

where k_a is the absorption rate constant expressed in $(\text{time})^{-1}$ units, which is equal to $P\cdot(SA)/V_{GI}$. In all pharmacokinetic text books, e.g. [18, 19] the classical analysis of one-

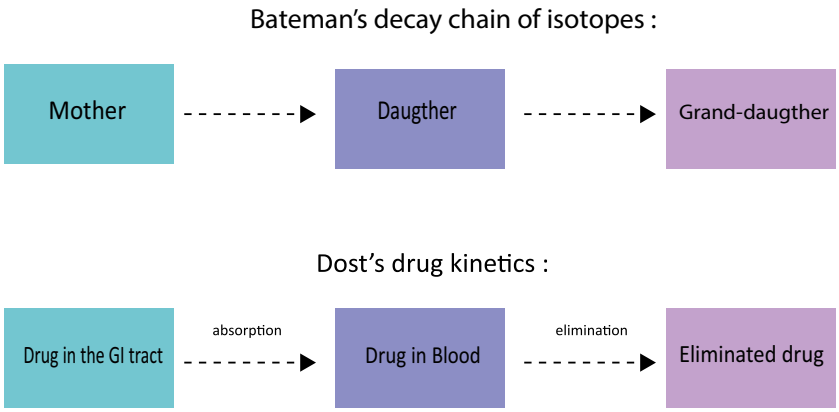

Fig. 1 Henry Bateman's vis a vis Friedrich Harmut Dost's kinetic considerations.

compartment model starts from Eq.4 assuming a first-order decrease of the amount of drug, A_{GI}:

$$\frac{dA_{GI}}{dt} = -k_a A_{GI} \tag{5}$$

which upon integration from t = 0, A_{GI}=FD to t = t, A_{GI} = A_{GI} one obtains:

$$A_{GI}(t) = FD \cdot e^{-k_a t} \tag{6}$$

Eq.6 is further coupled with the differential equation describing the change of drug concentration in blood, C_b, which eventually leads to Eq.1. Thus, the infinite absorption time implied from Eq.1 results from the first-order change (Eq.5) of the amount of drug in the gastrointestinal lumen, A_{GI}.

Development of Models Based on the Finite Drug Absorption Time Concept

One of the most important steps in oral drug absorption is the dissolution of drug in the gastrointestinal fluids [1, 3, 4, 18, 19]. In this context, we reconsider below the rate of drug permeation for the various drug classes (I-IV) [1, 3, 4] using the fundamental Eq.3 taking into account the dissolution process. Also, the pharmacokinetic considerations rely on one-compartment model disposition assuming for simplicity no first pass effect, i.e. fraction of dose absorbed = bioavailable fraction.

Class I Drugs

For highly soluble, highly permeable drugs (Class I), the rate of permeation is high, Eq.3, Fig. 2. Regardless the formulation administered (drug solution or solid formulation), these drugs do not exhibit either dissolution or permeability limited absorption. Therefore, the high value of P coupled with the high surface area, $(SA)_i$, of the small intestine lead to rapid and extensive absorption, Fig. 2. Therefore, this rapid absorption can be approximated with a constant rate of drug penetration:

$$(\textbf{\textit{Rate of Penetration}})_{\text{I}} = P \cdot (SA)_i \cdot (C_{GI}) = k_I = \frac{F_i D}{\tau_i} = \frac{D}{\tau_i} \tag{7}$$

where k_I denotes the constant penetration rate (mass/time units) for Class I drugs, F_i is the fraction of dose absorbed in the stomach and small intestine and τ_i is the duration of this initial absorption phase. Since Class I drugs are absorbed fully, $F_i = 1$ is being used in Eq.7. Accordingly, the change of drug blood concentration C_b as a function of time for Class I drugs is:

$$\frac{V_d dC_b}{dt} = k_I - k_{el} C_b V_d = \frac{D}{\tau_i} - k_{el} C_b V_d \tag{8}$$

Plausibly, the small intestine is the major site of absorption for Class I drugs while absorption always ceases in much shorter time than 4.86 h, which is the sum of gastric and small intestine transit time [17], Fig. 2. Eq.8 gives upon integration for t = 0, C_b = 0 and t = t, C_b = C_b:

$$C_b(t) = \frac{D}{\tau_i} \frac{1}{V_d k_{el}} \left(1 - e^{-k_{el} t}\right) \tag{9}$$

Upon completion of the absorption phase at time t = τ_i, the drug concentration will be $(C_b)_{\tau_i}$ in accordance with Eq.9. The change of drug concentration beyond time τ_i is described by the following equation

$$\frac{dC_b}{dt} = -k_{el}(C_b) \tag{10}$$

Eq.10 upon integration for t = τ_i, C_b = $(C_b)_{\tau i}$ and t → ∞, C_b = 0, leads to Eq.11 which describes the monotonic elimination phase

$$C_b(t) = (C_b)_{\tau_i} \cdot e^{-k_{el}(t-\tau_i)} \tag{11}$$

Class II Drugs

For low soluble, highly permeable drugs (Class II), the rate of drug permeation is low, Eq.3. This is so, since the maximum value of the term C_{GI}, of Eq.3 cannot be higher than the low

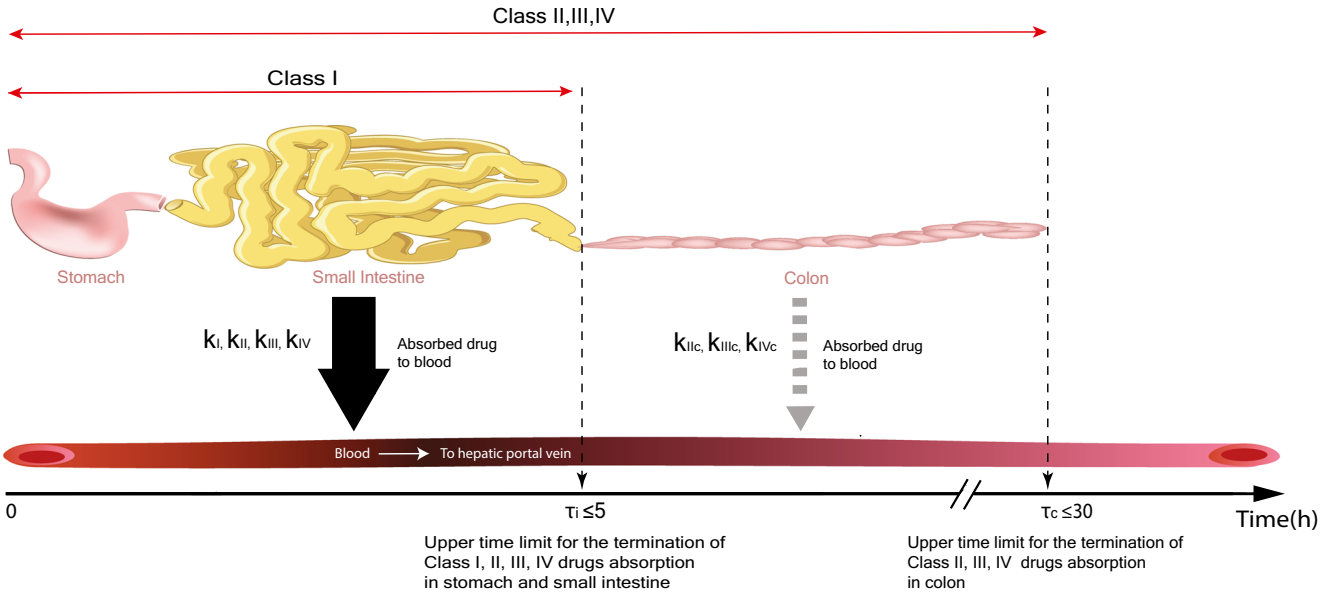

Fig. 2 A schematic of the biopharmaceutical/physiological drug absorption model, which relies on the transit times of the drug along the gastrointestinal tract. For Class I drugs, the completion of absorption ($F > 0.90$) ceases in a shorter time than the duration of the stomach and small intestine transit 4.86 h (21). For Class II, III and IV drugs, the limited overall absorption ($F < 0.90$) can be continued beyond the ileocecal valve and lasts not more than the whole gut transit time e.g. 29.81 h (21). The absorbed drug reaches the hepatic portal vein; the blood flow (20–40 cm/s) impose sink conditions on drug transfer. The thick black arrow denotes the major site of drug absorption, namely, the small intestine. The dashed arrow indicates the potentially limited drug absorption from the colon.

saturation solubility, C_S, of the drug in the gastrointestinal fluids. This solubility value can be also considered constant. Therefore, the rate of gastric and small intestine penetration for a Class II drug can be approximated.

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{II}} = P\cdot(SA)_i\cdot(C_S) = k_{II} = \frac{F_i D}{\tau_i} \qquad (12)$$

where k_{II} denotes the constant penetration rate (mass/time units) for Class II drugs, Fig. 2. Accordingly, the change of drug blood concentration C_b as a function of time assuming one-compartment model disposition for Class II drugs is

$$\frac{V_d dC_b}{dt} = k_{II} - k_{el} C_b V_d \qquad (13)$$

Eqs 12 and 13 roughly operate for not more than 4.86 h, which is the sum of gastric and small intestine transit time [17]. The passage of Class II drugs to the colon via the ileocecal valve, which separates the small intestine and the large intestine, can either result in the termination of drug absorption or the significant reduction of the rate of drug penetration since the effective surface area $(SA)_C$ is much smaller in the colon and the amount of unabsorbed drug at the ileocecal valve is equal to $(1\text{-}F_i)D$:

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{II},c} = P\cdot(SA)_C\cdot(C_S) = k_{II,c} = \frac{(1-F_i)D}{\tau_c-\tau_i}\cdot\lambda \qquad (14)$$

where τ_c denotes the termination time of drug absorption from the colon, λ is a coefficient $(0 < \lambda < 1)$ associated with the reduction of the penetration rate due to small surface area $(SA)_C$ compared to $(SA)_i$ and $k_{II,\,c}$ denotes the constant penetration rate (mass/time units) for Class II drugs in the colon, Fig. 2. Accordingly, the change of drug blood concentration C_b as a function of time assuming one-compartment model disposition for Class II drugs during the drug passage through the colon is

$$\frac{V_d dC_b}{dt} = k_{II,c} - k_{el} C_b V_d \qquad (15)$$

This equation roughly holds from 4.86 h to the time needed for the drug to reach the non-absorptive sites of the colon, τ_c, but certainly shorter than 20.28 or 31.95 h i.e. the colon transit time for a single-unit or multi-unit formulation, respectively [17], Fig. 2. At time τ_c absorption ceases; beyond this time point the drug is only eliminated from the body. Hence, the drug concentration decreases according to Eq.16, which is similar to Eq.11:

$$C_b(t) = (C_{b_c})_{\tau_c}\cdot e^{-k_{el}(t-\tau_c)} \qquad (16)$$

where $(C_{b_c})\,\tau_c$ is the drug concentration corresponding to time τ_c.

Class III Drugs

For highly soluble, low permeable drugs (Class III), the rate of drug permeation is low, Eq.3. This is so, since the low permeability value, P_l, is rate limiting for absorption; therefore, the

rate of penetration for a Class III drug, throughout the passage of drug from the stomach and small intestine, can be approximated:

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{III}} = P_l \cdot (SA)_i \cdot (C_{GI}) = k_{III} = \frac{F_i D}{\tau_i} \quad (17)$$

where k_{III} denotes the constant penetration rate (mass/time units) for Class III drugs, Fig. 2. Accordingly, the change of drug blood concentration C_b as a function of time for Class III drugs is

$$\frac{V_d dC_b}{dt} = k_{III} - k_{el} C_b V_d \quad (18)$$

Eqs 17 and 18 roughly operate for not more than 4.86 h, which is the sum of gastric and small intestine transit time [17]. The passage of Class III drugs to the colon via the ileocecal valve, can either result in the termination of drug absorption or the significant reduction of the rate of drug penetration since the effective surface area $(SA)_C$ is much smaller in the colon and the amount of unabsorbed drug at the ileocecal valve is equal to $(1 - F_i)D$:

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{III,c}} = P_l \cdot (SA)_C \cdot (C_{GI}) = k_{III,C} = \frac{(1-F_i)D}{\tau_c - \tau_i}\lambda \quad (19)$$

where $k_{III,C}$, denotes the zero-order penetration rate (mass/time units) for Class III drugs in the colon. Accordingly, the change of drug blood concentration C_b as a function of time assuming one-compartment model disposition for Class III drugs in the colon is

$$\frac{V_d dC_b}{dt} = k_{III,c} - k_{el} C_b V_d \quad (20)$$

This equation roughly holds from 4.86 h to the time needed for the drug to reach the non-absorptive sites of the colon, τ_C, but certainly shorter than 20.28 or 31.95 h i.e. the colon transit time for a single-unit or multi-unit formulation, respectively [21]. At time τ_c absorption ceases; beyond this time point the drug is only eliminated from the body. Hence, the drug concentration decreases according to Eq.16 for $t \geq \tau_c$.

Class IV Drugs

For low soluble, low permeable (Class IV) drugs, the rate of permeation is low, Eq.3. Both solubility and permeability are limiting absorption. The low values of the terms P and C_{GI} in Eq.3 allow their replacement, as explained above, with P_l and C_S, respectively. This leads to slow and limited absorption ($F << 0.90$). Therefore, this slow absorption can be approximated with a constant rate of penetration:

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{IV}} = P_l \cdot (SA)_i \cdot (C_S) = k_{IV} = \frac{F_i D}{\tau_i} \quad (21)$$

where k_{IV} denotes the constant penetration rate (mass/time units) for Class IV drugs, Fig. 2. Using the same syllogism delineated above, the differential equation describing the change of drug blood concentration C_b during the passage of drug from the stomach and small intestine (roughly, 4.86 h) [17] is as follows:

$$\frac{V_d dC_b}{dt} = k_{IV} - k_{el} C_b V_d \quad (22)$$

The passage of Class IV drugs to the colon via the ileocecal valve, can either result in the termination of drug absorption or the significant reduction of the rate of drug penetration since the effective surface area is much smaller in the colon $(SA)_C$ and the amount of unabsorbed drug at the ileocecal valve is equal to $(1 - F_i)D$:

$$(\textbf{\textit{Rate of Penetration}})_{\mathrm{IV,c}} = P_l \cdot (SA)_c \cdot (C_S) = k_{IV,c} = \frac{(1-F_i)D}{\tau_c - \tau_i}\lambda \quad (23)$$

where $k_{IV,C}$ denotes the constant penetration rate (mass/time units) for Class IV drugs in the colon. Accordingly, the change of drug blood concentration C_b as a function of time assuming one-compartment model disposition for Class IV drugs in the colon is

$$\frac{V_d dC_b}{dt} = k_{IV,c} - k_{el} C_b V_d \quad (24)$$

As explained above, this equation roughly holds from 4.86 h to the time needed for the drug to reach the non-absorptive sites of the colon, τ_c, (< 20.28 or < 31.95 h) i.e. the colon transit time for a single-unit or multi-unit formulation, respectively [17]. Beyond, this time point, τ_c, the drug is only eliminated from the body. Hence, the drug concentration decreases according to Eq.16 for $t \geq \tau_c$.

The theoretical section of oral drug absorption was based on i) the finite absorption time concept ii) the physiologically based transit times reported in the literature [17] and iii) the basic drug properties, namely, solubility and permeability, which have been adopted by the regulatory authorities as the key factors controlling oral drug absorption [3, 4]. However, the reader should be aware of the qualitative character of biopharmaceutics classification system, which implies large differences in the drug properties among the drugs of the same Class. Accordingly, the theoretical aspects developed here can be considered as a general framework of drug absorption while the *in vivo* drug behavior can vary remarkably even for drugs of the same Class [3, 4]. Moreover, deviations from the general modeling framework may be applied in accord with the experimental observations. For example, a drug may exhibit regional rate of absorption differences in the various segments of the small intestines e.g. jejunum and ileum. In such a case, two successive constant input rates can be considered.

Although the development of models was based on one compartment model disposition, similar equations can be written assuming two compartment model disposition [19]. For the purposes of the present work, we quote below the relevant equations assuming no absorption from the colon:

$$\frac{V_d dC_b}{dt} = k_1 - (k_{12} + k_{10})C_b V_d + k_{21} C_b V_d \quad for \quad 0 < t \leq \tau_i \tag{25}$$

$$\frac{dC_b}{dt} = -(k_{12} + k_{10})C_b + k_{21} C_2 \quad for \quad t > \tau_i \tag{26}$$

where k_{12}, k_{10}, k_{21} are the microconstants of the two-compartment model, C_2 the drug concentration in the peripheral compartment and k_1 can either be k_I or k_{II} or k_{III} or k_{IV} depending on the drug class type.

Due to the physiological relevance of the finite time absorption models developed, we coin the term physiologically based finite time pharmacokinetic (PBFTPK) models.

METHODS

Various simulation scenarios were used to generate (C_b, t) data for Class I, II, III and IV drugs. For Class I drugs, (C_b, t) curves were generated using Eq.9 and Eq.10 for $t \leq \tau_i$ and $t > \tau_i$, respectively assigning different values for D/τ_i ensuring rapid or very rapid and complete absorption (F = 1). For drug Classes II, III and IV, the (C_b, t)curves were generated using Eqs. 13, 15, or 18, 20 or 22, 24, respectively. Different values for the input rate parameters k_{II}, $k_{II,c}$, k_{III}, $k_{III,c}$, k_{IV}, $k_{IV,c}$, were used in order to study the shape of the (C_b, t) curves when drug absorption operates or not in the colon. In all cases, Eq.16 was used to simulate the elimination phase. All equations were integrated using Python's open-source SciPy v.1.4.1 ecosystem. Its embedded solvers using Adams'method for non-stiff problems provided us the continuous solutions of the first order differential equations. Data fitting was performed for four different drugs obtained from literature, namely, cephradine [20], ibuprofen [20], flurbiprofen [21] and itraconazole [22]. The PBFTPK models were fitted to the data using SciPy's *curve_fit* function. The function uses the Levenberg −Marquardt algorithm which has become a standard technique for nonlinear least squares problems [23]. The generated plots depict two discrete curves united at the top instead of one and undivided curve. The choice of binding the proposed pairwise equations with a Heaviside function [24] in order to erase discontinuities at C_{max} was also tested. Although it provided very competent R^2 metrics and almost identical fittings, the presentation of two discrete curves that join with a small discontinuity at their top, attributed to a small extrapolation at this data point due to the fitting procedure, was preferred. The selection of this format was navigated by the need to underline the two distinct physiological absorption and elimination processes. All corresponding plots were illustrated with SciPy's matplotlib.

RESULTS

Simulations

Figure 3 shows examples of Class I drugs with rapid (Fig.3a) and less rapid (Fig.3b) absorption. The two input rates are 133.33 and 50.0 *mg/h*, respectively. Figure 3c shows a very rapid drug absorption (*input rate* =1000 mg/*h*) in comparison with a set of curves generated from Eq.1 using different values for the absorption rate constant while in all cases a common value (0.1 h^{-1}) for the elimination rate constant is used. Visual inspection indicates that the plots exhibit only minor differences around the peak of the curves; accordingly, the justification of the operating model under *in vivo* conditions will be rather impossible since the fitted curves will be indistinguishable when data with experimental error are used.

Figure 4a shows examples of Class II or III or IV drugs assuming various input rates for drug absorption in the colon lasting 6 h while a common input rate 2.1 mg/*h* in the stomach/small intestine lasting 4 h has been used. The blue curve represents the most common case since no absorption is taking place in the colon. The green and purple curves, exhibit a monotonic decrease of drug concentration until time τ_c since the input rates in the colon (0.5 mg/*h*. and 1 mg/*h*, respectively) are much smaller than the input rate in the stomach/small intestine, 2.05 mg/*h*. The brown curve exhibits a sustained type profile since drug's absorption in the colon, (1.8 mg/*h*) has not been reduced drastically in comparison with the input rate (2.05 mg/*h*) in the stomach/small intestine. The orange curve represents the continuation of the drug's absorption in the colon for 6 more hours with imperishable input rate (2.1 mg/*h*).

Figure 4b is a representative example of a drug exhibiting regional permeability differences in the small intestine. Three successive input rates of 1.4, 1 and 0.8 mg/h in jejunum, ileum and colon, respectively have been simulated; the corresponding duration of the processes are 2, 1 and 7 h, respectively.

Data Analysis

Literature data of cephradine [20], ibuprofen [20] flurbiprofen [21] and itraconazole [22] were analyzed using the PBFTPK models developed. All (C_b, t) data belonging to the

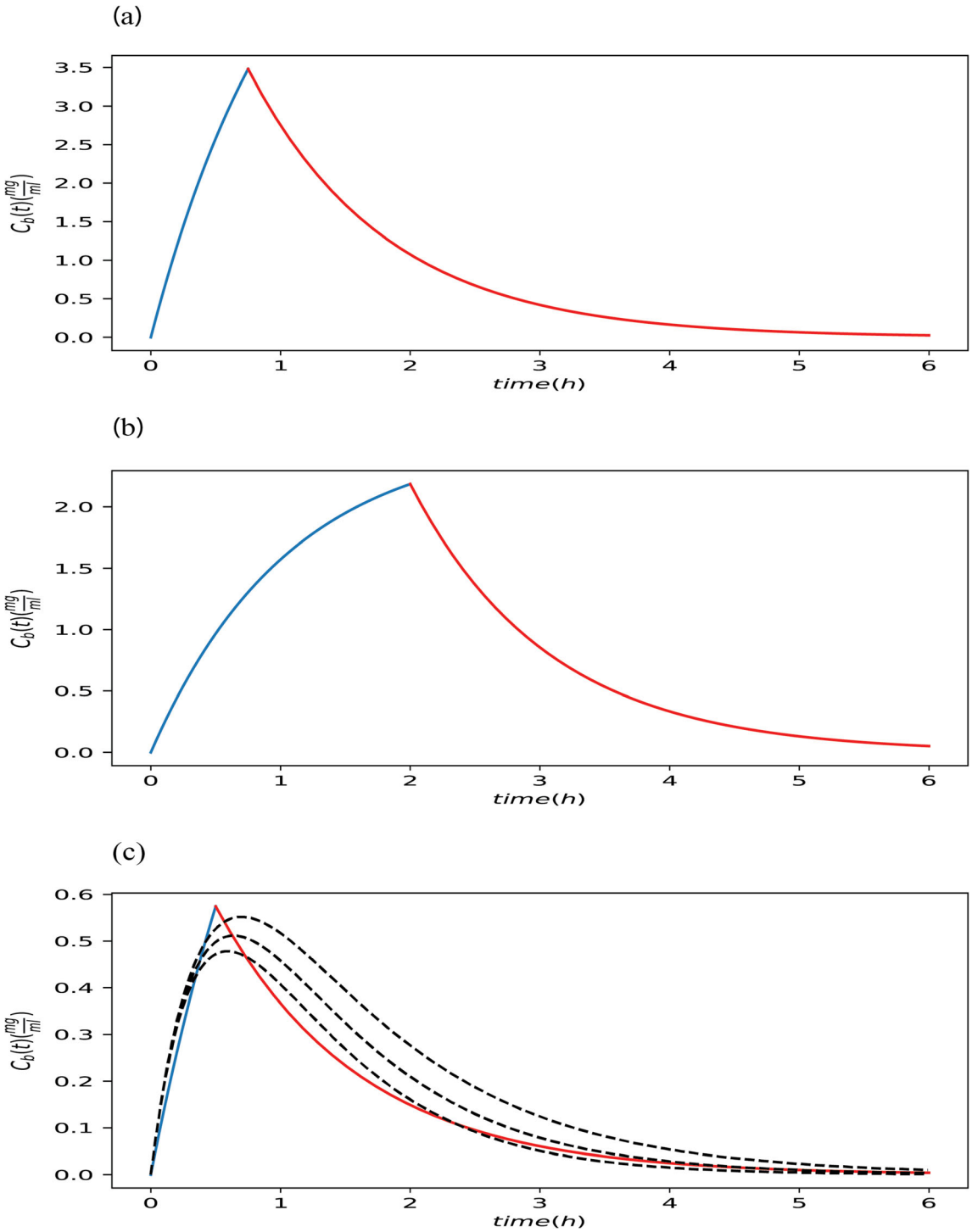

Fig. 3 (C_b, t) profiles for Class I drugs. Key: (a) Rapid drug absorption generated from Eqs. 9 and 11 adjusting D = 100 mg, τ_i = 0.75 h, V_d = 100 L, k_{el} = 0.1 h^{-1} (b) Less rapid absorption compared to (a) considering D = 100 mg, τ_i = 2 h, V_d = 100 L, k_{el} = 0.1 h^{-1}(c) Very rapid drug absorption (continuous line) considering D = 500 mg, τ_i = 0.5 h^{-1}, V_d = 680 L, k_{el} = 0.1 h^{-1} . The three dashed curves are generated from Eq. 1 using D = 500 mg, V_d = 680 L all sharing a common absorption rate constant k_a = 2.23 h^{-1} while k_{el} values from top to bottom are 0.85, 1.05, 1.25 h^{-1}, respectively. The blue lines correspond to drug absorption and the red lines represent the drug's elimination phase.

declining limbs of cephradine, ibuprofen and flurbiprofen profiles were analyzed using semi-logarithmic plots, Fig.5. For all sets of data very nice fittings were obtained with R^2 values 0.993, 0.989 and 0.980 while the elimination rate constants estimates were 0.97, 0.33 and 0.11 h^{-1} for cephradine, ibuprofen and flurbiprofen, respectively. The estimates of the respective elimination rate constants for cephradine, ibuprofen and flurbiprophen were coupled with Eq.13 and

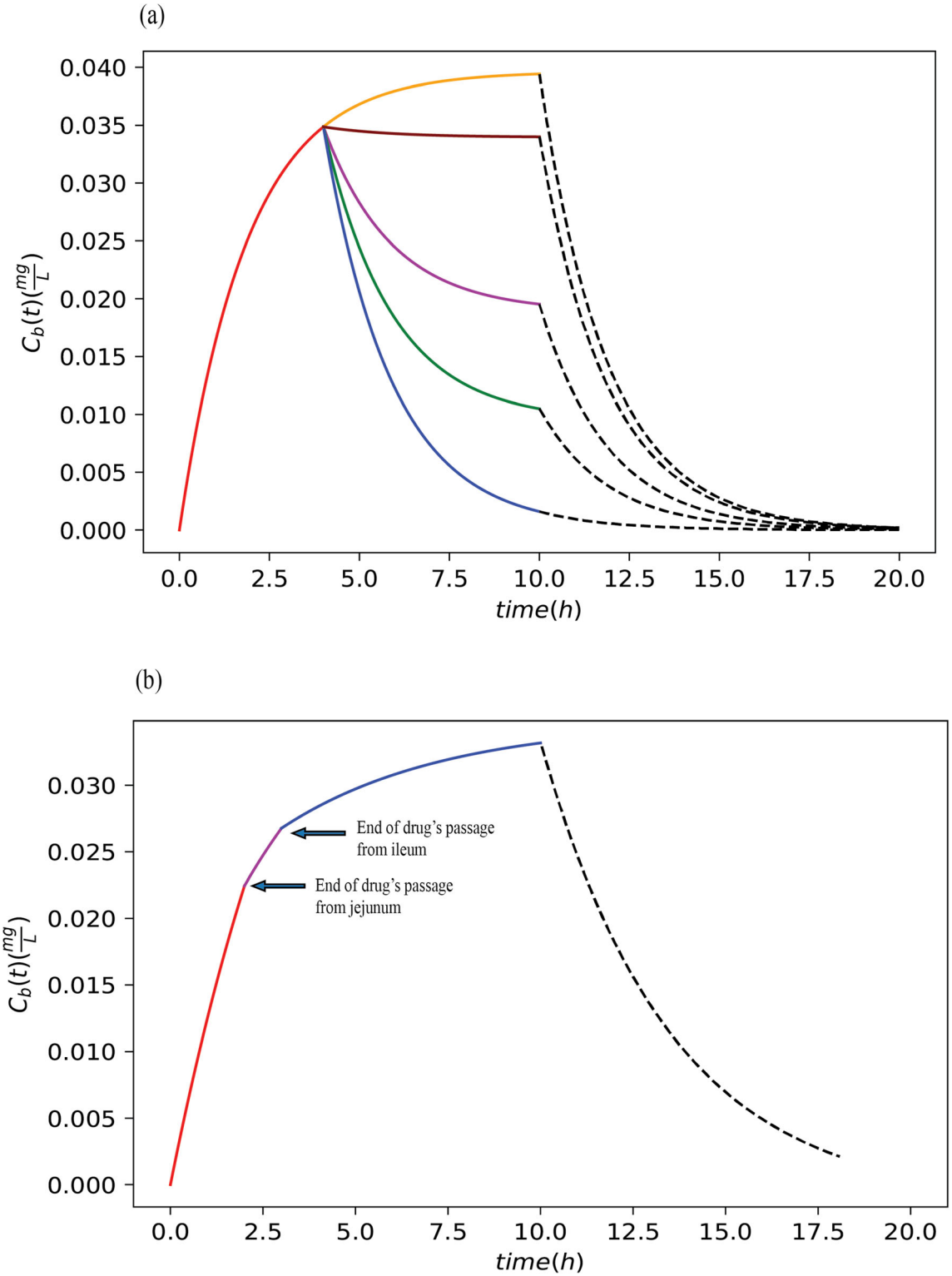

Fig. 4 (a) (C_b, t) curves for various drugs (Class II, or III, or IV) assuming a common i) elimination rate constant, $k_{el} = 0.53\ h^{-1}$ ii) small intestine input rate 2.1 mg/h and iii) volume of drug distribution 100 L. All drugs are absorbed in the stomach/small intestine where C_b reaches its peak at 4 h. After that point, absorption in the colon, which lasts 6 h, is observed for all coloured lines except for the blue line. Key: $k_{II,c}$, or $k_{III,c}$, or $k_{IV,c}$ 2.05, 1.80, 1.00, 0.50, 0 mg/h from top to bottom, respectively (b) (C_b, t) curve for a drug exhibiting three successive constant rates of absorption 1.4, 1.0, 0.8 mg/h in the jejunum, ileum and colon, respectively. The duration of absorption is 2, 1 and 7 h, respectively. The arrows indicate the termination of absorption at the end of the two segments of the small intestine. The drug dose is equal to 100 mg, $k_{el} = 0.23\ h^{-1}$, while the volume of drug distribution is equal to 100 L.

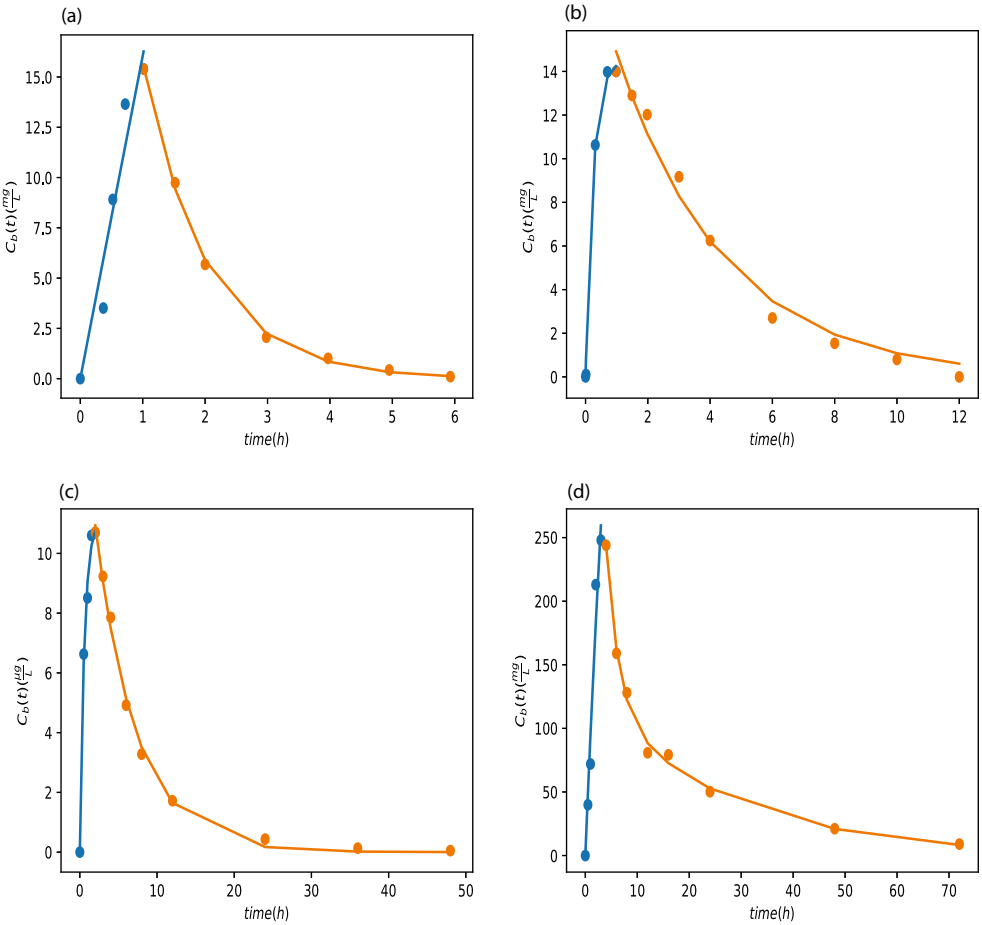

Fig. 5 Curve fitting of Eqs. (25, 26) to all data points. Key: (a) Cephradine. Dose = 500 mg, $R^2 = 0.9723$. (b) Ibuprofen, Dose = 200 mg, $R^2 = 0.9961$(c) Flurbiprofen, Dose = 100 mg, $R^2 = 0.9908$. (d) Itraconazole, Dose = 200 mg, $R^2 = 0.9797$. The blue lines correspond to drug absorption and the orange lines represent the drug's elimination phase.

fitted to the entire set of data, Fig.6a-c. The R^2 values of the fittings were 0.9723, 0.9961 and 0.9908 while the estimates for optimal k_{II} were 15.07, 11.12 mg/h for ibuprofen and flurbiprophen, respectively. A meaningless estimate was derived for cephradine input rate upon curve fitting. This is attributed to the very steep change of cephradine concentration in the absorption phase. A graphical estimate was derived from the data points assuming a linear change of 14.81, mg/*h*. Since itraconazole (26) is a two-compartment model, Class II drug [24] the entire set of the declining limb of concentration time data was analyzed using the general equation that describes a two-compartment disposition model (19):

$$C_b = Ae^{-at} + Be^{-\beta t} \tag{27}$$

The estimates for the constants A, B, α, β were computed with a nonlinear least-squares approach using the Levenberg −Marquardt algorithm [24]. A nice fitting was obtained, $R^2 = 0.9768$, Fig.5d; the estimates for A, B, α, β were 679.46 mg/L, 13.26 mg/L, 0.41 h^{-1}, 0.038 h^{-1}, respectively. Subsequently, estimates for the microconstants, k_{12}, k_{10} k_{21} were derived algebraically [19] and used in a curve-fitting exercise of Eqs. 25 and 26 to the entire set of itraconazole experimental data, Fig.6d. The algorithm converged with an R^2 metric 0.9797, while the estimate for k1 was 990.07 mg/h..

The analysis of all data demonstrates that the absorption of drugs has been terminated at 1.0, 2.0, 0.7, 3.0 h for cephradine, ibuprofen, flurbiprofen and itraconazole, respectively, Fig.6. In other words, all drugs were absorbed in the small intestine, which is in accord with the general and common wisdom belief.

DISCUSSION

The physiological aspects of the PBFTPK models rely on the physiological/anatomical differences of the two regions, small intestine and large intestine. It is widely known today that because of its permeability, large surface area and high blood flow, the small intestine is the primary site for drug absorption, Fig.2. In fact, a monolayer of enterocytes that is characterized by protrusions that extend into the gut lumen, called villi, result in a potential absorptive surface area of $60m^2$ in both the jejunum and ileum [25]. On the contrary, the colon surface area totals around 0.25 m^2 as there are no villi [26]. This huge anatomical difference causes a very large difference in the rate

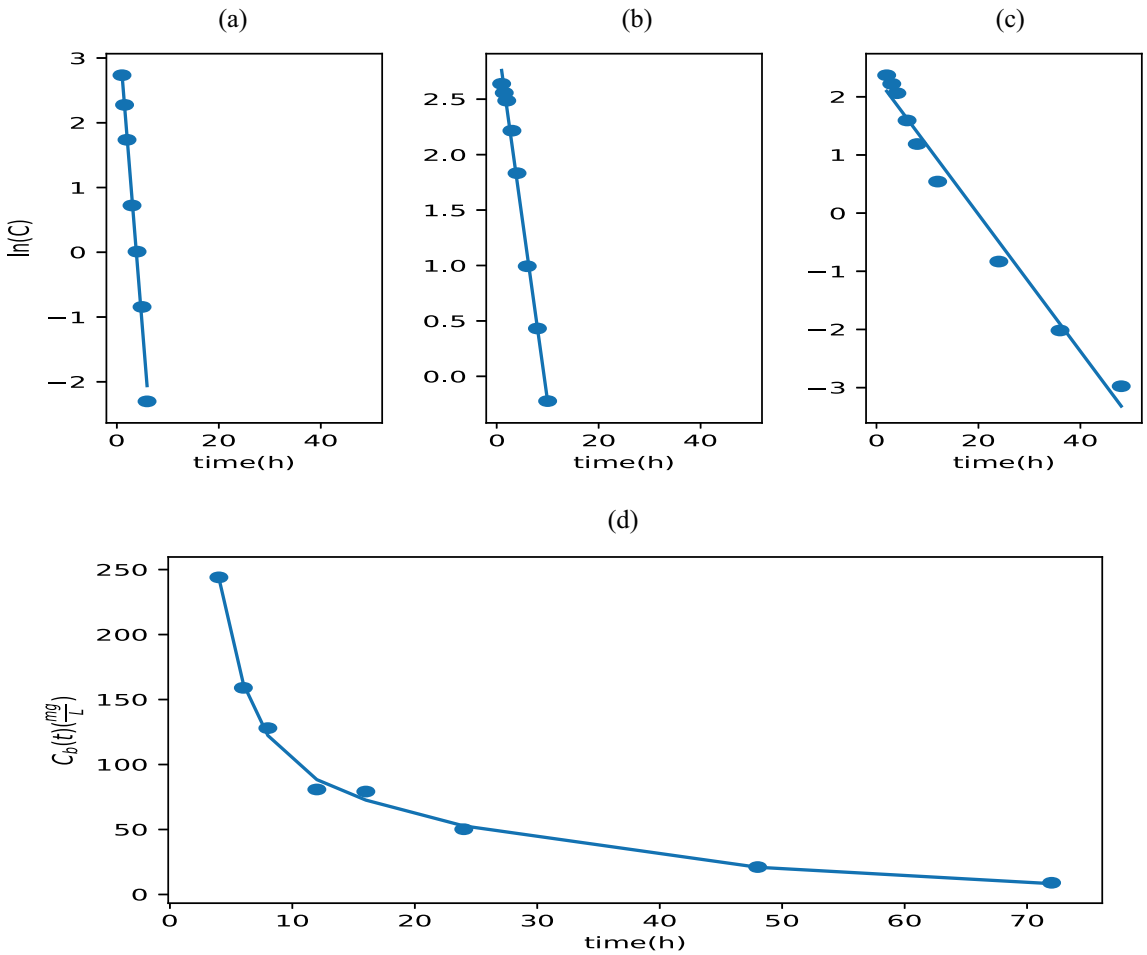

Fig. 6 Computation of the slope of one-compartment model drugs [(a), (b), (c)] using semilogarithmic plots for all data points of the declining limb of the (C_b, t) curve. Key: (a) Cephradine, Dose = 500 mg, $k_{el} = 0.97\ h^{-1}$,$R^2 = 0.991$; (b) Ibuprofen, Dose = 200 mg, $k_{el} = 0.33\ h^{-1}$, $R^2 = 0.993$; Flurbiprofen, Dose = 100 mg, $k_{el} = 0.11\ h^{-1}$, $R^2 = 0.980$. (d) Non-linear two-compartment model fit to all itraconazole data points of the declining limb of the (C_b, t) curve of a dose 200 mg. Computation of optimal parameters: A = 679.46 mg/ mL, $\alpha = 0.415\ h^{-1}$, B = 132.68 mg /mL, $\beta = 0.038\ h^{-1}$ from all elimination phase data points, $R^2 = 0.9985$ (e) Phase plane plot (29) of itraconazole computed for the last three data points of the β-phase data; Intercept = −0.012 mg /mLh^{-1}, Slope = $0.032\ h^{-1}$.

of drug absorption, Eqs. 7,12,14,21. Besides, drug's transport from the gastrointestinal lumen to the portal vein relies on the sink conditions' principle of the universally accepted first-order drug absorption notion. This is substantiated by the fact that the blood in the portal vein has a velocity of 20–40 cm/s [27], which does not allow Fick's reversibility considerations for the drug transfer. In parallel, the small intestine was presented (Fig.2) as a homogeneous compartment in terms of drug's uptake. However, drug absorption takes place mainly from the lower part of the small intestine. For example, drug absorption can be higher from the jejunum than the ileum. A relevant simulation is shown in Fig.4b whereas the initial absorption rate from the jejunum is 1,4 mg/h and lasts two hours followed by an absorption rate of 1.0 from the ileum for 1 h while the rate of drug absorption in the colon is equal to 0.8 mg/h and lasts 7 h.

The unique features of the PBFTPK models are the finite termination times for the absorption phases, τ_i and τ_c, respectively. The upper limit for τ_i is 5 h, Fig.2, with most frequently observed values in the literature in the range 1–3 h depending on the drug's biopharmaceutical properties. The upper limit for τ_c is 30 h, Fig.2, while the most usual values for τ_C are unknown since estimates for τ_c have not been explored so far. However, a large number of *in vivo* studies based on imaging techniques like gamma scintigraphy or magnetic resonance imaging coupled with drug blood measurements have shown that the completion of the absorption phase is terminated during the drug's passage from the small intestine e.g. erythromycin study [28].

Needless to say that according to the current theory (Eq.1) the termination of either the elimination or the absorption phase is irreconcilable [13–16].

Simulations

The simulations of Fig.3 lead to important observations for Class I drugs. First of all, there are conceptual differences in

the concentration, time maxima generated from the PBFTPK models and the corresponding parameters adhering to the Bateman equation. For Eq.1, Cmax corresponds to the steady state concentration derived from the classical equality (Rate in = Rate out) [19]; this applies to tmax too [19]. On the contrary, the pair $((C_b)_{\tau_i}, \tau_i)$ denotes the end of drug's absorption phase i.e. for a Class I drug no more drug is available for absorption. The value of τ_i for Class I drugs reflects the high values of the biopharmaceutical properties solubility and permeability; in theory, the smaller the value of τ_i, the higher "the Class I character" of drug. Besides, the initial absorption phase can be almost linear (Fig.3a) or nonlinear (Fig.3b) depending on the magnitude of the elimination rate constant in comparison with the magnitude of the input rate. In parallel, the declining limbs for all PBFTPK models concave upwards and represent a single elimination phase. On the contrary, the declining limb of the curves generated from Eq.1 concave downwards initially and then upwards beyond the inflection point at $2t_{max}$ [13]. However, the similarity of the curves is remarkable.

For drug Classes II, III, and IV, drug absorption beyond the small intestine can be considered. During the time period τ_i - τ_c, the identical form of Eqs. 15, 20 and 24 for drug Classes II, III, and IV, respectively allows a common consideration. The (C_b, t) profiles during the time interval τ_i - τ_c is heavily dependent on the relative magnitude of the two terms at the right hand side of Eqs. 15, 20 and 24. Obviously, during the time interval τ_i - τ_c, the derivative of Eq. 15 or 20 or 24 is constantly negative for the three monotonically decreasing profiles at the bottom of Fig.4a. The bottom curve of Fig.4a represents the most common case whereas drug absorption is not observed in the colon and τ_c becomes meaningless since drug absorption is terminated in the small intestine ($\leq\tau_i$). For the top curve of Fig.4, the derivative of Eqs. 15, 20 and 24 is initially positive, becomes equal to zero at pseudo steady state and then negative beyond time τ_c (10 h in the example of Fig.4a). For all examples depicted in Fig.4a, a common elimination phase (generated from Eq.16, $t \geq \tau_c$) is shown. It can be seen that a single elimination phase prevails during the time course of drug in the body beyond τ_i only for the bottom curve. Besides, the separation of the elimination phase cannot be easily accomplished under *in vivo* conditions in several cases e.g. the second from the bottom curve of Fig.4a. Moreover, multiple regional input rates can be considered if a prolonged absorption phase is observed, Fig.4b. Overall, this analysis reveals that rich kinetics can be encountered for Class II, III, and IV drugs, which is dependent on the rate of drug absorption in the colon.

In all cases, the amount of drug remaining in the body $A\tau_c$, at time τ_c, is

$$A_{\tau_C} = D - F_i D - F_c D = D(1 - F_i - F_c) \tag{28}$$

where F_i and F_C are the fractions of dose absorbed until time τ_i and during the time interval τ_i - τ_c, respectively.

Data Analysis

The results presented in Fig.5a-d deliver a very important message. For the four drugs examined, their absorption has been completed while the drugs are still in the small intestine and the declining limb of the (C_b, t) curves represents a single elimination phase. This is so, since in all cases the τ_i values are smaller than 3 h, Fig.2 & Fig.6; moreover, a phase plane plot Fig.5e [29] for itraconazole was constructed to verify that the β-phase data of itraconazole represent the terminal elimination phase. The y-intercept estimate (mean ± 2SD) overlaps the origin (0,0) of the phase plane plot axes [29], which clearly indicates that the β-phase data of itraconazole represent a single-terminal elimination phase. The completion of absorption in the small intestines for all drugs examined does not mean that the drugs have been fully absorbed; it means that their limited absorption has been terminated during their passage from the small intestine. This is in accord with their biopharmaceutical classification since ibuprofen, flurbiprofen and itraconazole belong to Class II while cephradine has been classified as Class I or III drug. Although the termination of all four drugs in the small intestine is physiologically sound, its significance for many more drugs has to be tested in a very large sample of various drugs using the PBFTPK concepts.

Implications

The parameters of the PBFTPK models are directly linked with the fundamental drug properties of solubility and permeability, Eqs. 7, 12, 17, 21. Therefore, upon their estimation the investigator will get a quantitative measure in meaningful mg/h units for comparative purposes among drug classes in the context of biopharmaceutical classification system [1, 3, 4]. Work is in progress towards the analysis of a large set of drug data from various biopharmaceutical classes in order to find correlations, if any, between the input rate estimates and the solubility and permeability of drugs. Moreover, correlations between the estimates for τ_i and τ_c and the biopharmaceutical classification of drugs will be explored since the estimates for τ_i and τ_c have clear physiological meaning, Fig.2. On the opposite, the currently used absorption rate constant k_a (Eq.1) has no clear biological meaning. Thus, in an interspecies pharmacokinetic study is difficult to decipher how an animal estimate for k_a scales to humans; the biopharmaceutical/physiological basis of the parameters of PBFTPK models will greatly facilitate the choice of parameter values for the prediction of human pharmacokinetics from animal data. Some of the most serious implications of PBFTPK models in different areas of research are

summarized below assuming either limited or non-existent drug absorption in the colon.

First, several aspects of regulatory science associated with bioavailability, bioequivalence and biowaiver issues [3–5] should be re-considered in the light of PBFTPK models. Since drug absorption ceases at either time τ_i or τ_c, the corresponding areas $AUC_{0-\tau i}$, $AUC_{0-\tau c}$ are related to the amount of drug absorbed until the end of absorption process. In parallel, the areas $AUC_{\tau i-\infty}$, $AUC_{\tau c-\infty}$ beyond time τ_i or τ_c are related to the absorbed drug amount remaining in the blood at time τ_i and τ_c, respectively. In this vein, the ratio $(AUC_{\tau c-\infty})/(AUC_{0-\infty})$ corresponds to the fraction of the bioavailable dose remaining in the general circulation at time τ_c (completion of the absorption process). In view of these observations, concern is arising for the relevant recommendation of the current bioequivalence guidelines (3),(4), namely, "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 $AUC_{0-\infty}$". Alternatively, the bioequivalence guidelines [3, 4] propose a specific time limit, 72 h, for the calculation of total AUC as follows, "AUC truncated at 72h (AUC_{0-72}) may be used as an alternative to AUC0-t for comparison of extent of exposure as the absorption phase has been covered by 72h for immediate release formulations." Plausibly, drug agencies have to assess the impact of the present work not only on the above recommendations but also on the general regulatory aspects of bioequivalence and biowaiver studies, absolute bioavailability and rate exposure metrics (partial areas, [20, 30, 31]).

Second, a large number of studies e.g. *in vitro, in vivo correlations* (IVIVC), interspecies pharmacokinetic scaling, studies for the determination of first in human dose, paediatric pharmacokinetic scaling studies for the determination of the paediatric dose rely on the proper pharmacokinetic analysis of data as well as on estimates of exposure metrics e.g. areas under the plasma concentration-time curve. Obviously, the results of the present study can be applied in the above fields of research and enhance the predictive power of the relevant studies. Finally, the PBFTPK models can be combined with pharmacodynamic (PD) models leading to new PK-PD applications; besides, pharmacometric approaches can utilize as absorption parameters τ_i and τ_c while $AUC_{\tau i-\infty}$ and $AUC_{\tau c-\infty}$ can be used as parameters for the elimination characteristics. Relevant covariates associated with either absorption or elimination can be reconsidered in the light of the present study.

Third, the current work was based on linear kinetics using one-or two-compartment model drug disposition. However, the PBFTPK models can be also applied to drugs following nonlinear, Michaelis Menten disposition kinetics [19]. For purposes of completion, the importance of uptake and efflux transporters identified in the intestines as well as in the liver, kidney and blood brain barrier should be mentioned. Although carrier mediated transport relies on Michaelis-Menten kinetics and is associated with the active transporters, passive diffusion aspects are also linked with passive transporters e.g. BBB-shuttles that cross the BBB by a passive transport mechanism [32, 33].

CONCLUSIONS

This study shows that oral drug absorption is terminated in finite time. This was achieved using the physiologically relevant first-order under sink conditions absorption principle coupled with the rate-limiting role in drug absorption of the BCS Class dependent parameters solubility and permeability. The PBFTPK models developed rely on the finite time absorption concept. Our results are very promising for the use of PBFTPK models in a variety of applications in pharmaceutical research.

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REFERENCES

1. Amidon GL, Lennernäs H, Shah VP, Crison JR. "A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability". In: *Pharm Res* 12.3 (1995), pp. 413–20. [Journal Article].
2. Wu C, Benet L. "Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system". In: *Pharm Res* 22.1 (2005), pp. 11–23. [Journal Article].
3. 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. [Scientific Guideline].
4. European Medicines Agency. Committee for medicinal products for human use (CHMP) Guideline on the investigation of bioequivalence. London, Jan. 2017.[Scientific Guideline].
5. Charalabidis A, Sfouni M, Bergström C, Macheras P. "BCS and BDDCS: Beyond guidelines". In: *International Journal of Pharmaceutics* (2019), 566:264–281. [Journal Article].
6. Macheras P, Karalis V, Valsami G. "Keeping a critical eye on the science and the regulation of oral drug absorption: a review". *Journal of Pharmaceutical Sciences*102, 3018–3036 (2013).[Journal Article].
7. Yu LX and Amidon GL (1999) "A compartmental absorption and transit model for estimating oral drug absorption". In: *Int J Pharm* 186:119-125. [journal article].

8. Lin L, Wong H. "Predicting Oral Drug Absorption: Mini Review on Physiologically-Based Pharmacokinetic Models". In: *Pharmaceutics*. 2017;9(4):41. [Journal Article].
9. Chung J, Kesisoglou F. "Physiologically Based Oral Absorption Modelling to Study Gut-Level Drug Interactions". In: *J Pharm Sci*.2018;107(1):18–23. [Journal Article].
10. Stillhart C, Pepin X, Tistaert C, et al. "PBPK Absorption Modeling: Establishing the *In Vitro-In Vivo* Link-Industry Perspective". In: *AAPS J*.2019;21(2):19. [Journal Article].
11. Rinaki E, Dokoumetzidis A, Valsami G, Macheras P. "Identification of biowaivers among class II drugs: theoretical justification and practical examples". In : *Pharmaceutical Research*.21, 1567–1572 (2004). [Journal Article].
12. Macheras P, Karalis V. "A non-binary biopharmaceutical classification of drugs : the ABΓsystem". In: *International Journal of Pharmaceutics* 464 (2014), pp. 85–90. [Journal Article].
13. Macheras P. "On an Unphysical Hypothesis of Bateman Equation and its Implications for Pharmacokinetics". In: *Pharmaceutical research* 36 (July 2019), p. 94. [Journal Article].
14. Dost F. H. "Der Blutspiegel. Kinetik der Konzentrationsverläufe in der Kreislaufflüssigkeit". In: Leipzig (Sept. 1953). [Journal Article].
15. H. Bateman. "Solution of a system of differential equations occurring in the theory of radioactive transformations". In: *Proc. Cambridge Philos. Soc.* 15 (1910), pp. 423–427. [Journal Article].
16. Garrett Edward R. "The Bateman function revisited: A critical reevaluation of the quantitative expressions to characterize concentrations in the one compartment body model as a function of time with first-order invasion and first-order elimination". In: *Journal of Pharmacokinetics and Biopharmaceutics*, 22.**2** (Apr. 1994), pp. 103–128. [Journal Article].
17. Abuhelwa A, Foster D, Upton R. "A Quantitative Review and Meta-models of the Variability and Factors Affecting Oral Drug Absorption-Part II: Gastrointestinal Transit Time". In: *AAPS J* 18.5 (Sept. 2016), pp. 1322–1333. DOI: https://doi.org/10.1208/s12248- 016- 9953- 7. [Journal Article].
18. Rowland M, Tozer TN. Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications. Wolters Kluwer Health/Lippincott William Wilkins, 2011. [Book].
19. Niazi S. Textbook of biopharmaceutics and clinical pharmacokinetics.Chapter 7. 292 Madison Ave., New York: Appleton-Century-Crofts, 1979. [Book].
20. Chen ML. "An Alternative Approach for Assessment of Rate of Absorption in Bioequivalence Studies". In: *Pharmaceutical Research* 9.11 (Apr. 1992). [Journal Article].
21. Szpunar GJ, Albert KS, Bole GG, Dreyfus JN, Lockwood GF, Wagner JG. "Pharmacokinetics of flurbiprofen in man I. Area/dose relationships". In: *Biopharmaceutics Drug Disposition* 8 (1987), pp. 273–283. [Journal Article].
22. Hardin TC, Graybill JR, Fetchick R, Woestenborghs R, Rinaldi MG, Kuhn JG. "Pharmacokinetics of Itraconazole following Oral Administration to Normal Volunteers". In: *Antimicrobial Agents and Chemotherapy* 32.9 (Dec. 1988), pp. 1310–1313. [Journal Article].
23. Arfken GB, Weber HJ, Harris. Mathematical Methods for Physicists. Seventh Edition. 225 Wyman Street Waltham MA 02451 USA: Elsevier, 2013. [Book].
24. Press WH, Teukolsky SA, Vetterling WT, Flannery BP. Numerical Recipes 3rd Edition: The Art of Scientific Computing. Cambridge University Press, 2007. [Book].
25. Vertzoni M, Augustijns P, Grimm M, Koziolek M, Lemmens G, Parrott N, Pentafragka C, Reppas C, Rubbens J, Van Den Abeele J, Vanuytsel T, Weitschies W, Wilson CG. "Impact of regional differences along the gastrointestinal tract of healthy adults on oral drug absorption: An UNGAP review". In: *Eur J Pharm Sci* 134 (June 2019), pp. 153–175. DOI: https://doi.org/10.1016/j.ejps.2019.04.013. [Journal Article].
26. Kararli T, Searle GD. "Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals". In: *Biopharmaceutics and Drug Disposition* 16 (1995), pp. 351–380. DOI: https://doi.org/10.1002/bdd.2510160502. [Journal Article].
27. Iranpour P, Lall C, Houshyar R, Helmy M, Yang A, Choi JI, Ward G, Goodwin SC. "Altered Doppler flow patterns in cirrhosis patients: an overview". In: *Ultrasonography* 35.1 (Jan. 2016), pp. 3–12. DOI: https://doi.org/10.14366/usg.15020. [Journal Article].
28. Digenis GA, Sandefer EP, Parr AF, Beihn R, McClain C, Scheinthal BM, Ghebre-Sellassie I, Iyer U, Nesbitt RU, Randinitis E. "Gastrointestinal behavior of orally administered radiolabeled erythromycin pellets in man as determined by gamma scintigraphy". In: *Journal of Clinical Pharmacology* 30.7 (July 1990), pp. 621–31. [Journal Article].
29. Dokoumetzidis A, Iliadis A, Macheras P. "An alternative method for the estimation of the terminal slope when a few data points are available". In: *Journal of Pharmaceutical Sciences* 88 (Apr. 1999), pp. 557–560. [Journal Article].
30. Macheras P, Dokoumetzidis A. "On the use of partial AUC as an early exposure metric". In: *European Journal of Pharmaceutical Sciences* 10 (2000), pp. 91–5. [Journal Article].
31. Macheras P, Symillides M, Reppas C. "The cut-off time point of the partial area method for assessment of rate of absorption in bioequivalence studies". In: *Pharmaceutical Research* 11 (1994), pp. 831–834. [Journal Article].
32. Sanchez-Navarro M, Teixido M, Giralt E. "Jumping hurdles: peptides able to overcome biological barriers". In: *Acc Chem Res* 50.8 (2017), pp. 1847–1854. DOI: 10 . 1021 / acs. accounts. 7b00204. [Journal Article].
33. Macheras P, Iliadis A, Melagraki G. A reaction limited in vivo dissolution model for the study of drug absorption: Towards a new paradigm for the biopharmaceutic classification of drugs. Eur J Pharm Sci. 2018;117:98–106.

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