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Introducing logistic enzyme kinetics

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For treatment of in vitro enzyme kinetics the Michaelis-Menten equation is generalized to a logistic form. From the new probabilistic viewpoint the classical Michaelis-Menten kinetic resembles the first order expansion of the logistic one with respect to the bound substrate concentration. The probabilistic approach has three advantages. First, it better describes the quasi steady state approximation of catalysis. Second, it substitutes a logistic analytical solution for the closed-form *W*-Lambert solution for the progress curves of the substrate decay or product formation, this way recovering the previously introduced ansatz by M. V. Putz, A.-M. Lacrămă and V. Ostafe *Int. J. Mol. Sci.* **7**, 439 (2006). Finally, it provides an alternative time-dependent fitting curve for estimating kinetic parameters that replaces the earlier linear plot representation with a first order of time expansion.

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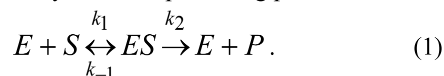
1. Introduction

It has long been recognized that the chemical reactions that support life are mediated by enzymes and their kinetics. Brown [1, 2] and Henri [3, 4] first proposed that enzyme catalysis is based on the reversible reaction between and enzyme E and a substrate S with rate constant k_1 to form an intermediate enzyme-substrate complex ES. The complex then reacts irreversibly with rate constant k_2, regenerating the enzyme E and producing product P:



$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P . \quad (1)$$

The mathematical formulation of this process was developed by Michaelis-Menten [5] and is represented by the classical Michaelis-Menten equation (2) where $V_{max} = k_2[E_0]$, and $K_M = (k_{-1} + k_2)/k_1$.

$$\left(-\frac{d}{dt}[S](t)\right)_{t=0} = v_0 = \frac{V_{max}[S_0]}{[S_0] + K_M} \quad (2)$$

Solution of the Michaelis-Menten equation to estimate the kinetic constants has traditionally involved linear transformation [6, 7] or the use of graphical methods [8-10] both of which are subject to error. For example, when the double reciprocal linear plot of equation (2) is used, small errors in $[S_0]$ or v_0 lead to large errors in $1/[S_0]$ or $1/v_0$ and thus to large errors in K_M and V_{max} [11, 12]. On the other hand, direct application of equation (2) requires an estimate of the initial velocity for every point to be fitted to the progress curve [13, 14].

Integration of equation (2) provides an instantaneous version of the Michaelis-Menten equation [15, 16] as equation (3), which also has equivalent linear forms for a number of different cases [17].

$$V_{max} t = \left([S_0] - [S](t)\right) - K_M \ln\left(\frac{[S](t)}{[S_0]}\right) \quad (3)$$

Working formulas developed for particular cases such as enzyme inhibition, by Duggleby and Morrison [18], multiple substrates, by Duggleby and Wood [19], the presence of an inhibitor at concentrations comparable to the enzyme concentration, by Szedlacsek et al. [20], and gradual inactivation of an enzyme, by Duggleby [21], ignore the fact that the largest experimental error is in the concentration and not in the velocity. Because of this, the linear transformations and approximations distort the experimental errors, leading to possible bias in the estimates of K_M and V_{max} [14]. Schnell and Mendoza [13] derived the closed form solution (4) of the instantaneous Michaelis-Menten equation in terms of the *W*-Lambert function [22].

$$[S]_W(t) = K_M W\left(\frac{[S_0]}{K_M} e^{\frac{[S_0] - V_{max} t}{K_M}}\right) \quad (4)$$

Relationship (4) is valid at all times and makes it possible to treat many complex enzymatic interactions mathematically, extensively studied by Schnell and Mendoza [14, 22, 23]. This model is, however, limited because the *W*- function is not widely available in curve-fitting software.

More generally, it has been shown that the computational methods used to numerically integrate the instantaneous Michaelis-Menten equation are time-consuming and relatively slow [24-26].

In the present work we propose a generalized version of the classical Michaelis-Menten equation that avoids most of the difficulties encountered in modeling enzymatic kinetics in vitro.

2. Theoretical model

In the post genomic era the development of kinetic models that allow simulation of complicated metabolic pathways and protein interactions is becoming increasingly important [27,28]. Unfortunately, the difference between an *in vivo* biological system and homogeneous *in vitro* conditions is large, as shown by Schnell and Turner [29]. Mathematical treatments of biochemical kinetics have been developed from the law of mass action *in vitro* but the modifications required to bring them in line with the stochastic *in vivo* situation are still under development [30-32].

We use a probabilistic approach, based on the law of mass action, to characterize *in vitro* enzymatic reactions of type (1):

$$1 = P_{\mathrm{REACT}}([S]_{bind}) + P_{\mathrm{UNREACT}}([S]_{bind}) . \quad (5)$$

In equation (5), $P_{\mathrm{REACT}}([S]_{bind})$ is the probability that the reactions (1) proceed at a certain concentration of substrate binding to the enzyme $[S]_{bind}$. The limits are:

$$P_{\mathrm{REACT}}([S]_{bind}) = \begin{cases} 0 \ , & [S]_{bind} \to 0 \\ 1 \ , & [S]_{bind} >> 0 \end{cases} \quad (6)$$

$P_{\mathrm{REACT}}([S]_{bind}) = 0$ when the enzymatic reaction does not proceed or when it stops because the substrate fails to bind or is entirely consumed. Conversely, $P_{\mathrm{REACT}}([S]_{bind}) = 1$ when the enzymatic reaction proceeds, and it is related to the standard quasi-steady-states approximation (QSSA). The probability of the occurrence of products in reactions (1) lies between these limits. Similarly, in the case where enzymatic catalysis does not take place, $P_{\mathrm{UNREACT}}([S]_{bind})$, the limits are:

$$P_{\mathrm{UNREACT}}([S]_{bind}) = \begin{cases} 1 \ , & [S]_{bind} \to 0 \\ 0 \ , & [S]_{bind} >> 0 \end{cases} \quad (7)$$

This probabilistic treatment of enzymatic kinetics is based on the chemical bonding behavior of enzymes that act upon substrate molecules through diverse mechanisms and it may offer the key to the quantitative treatment of different types of enzyme catalysis [33].

To expand the terms of equation (5) to analyze reactions in the (1) we first recognize that the binding substrate concentration can be treated as the instantaneous substrate concentration: $[S]_{bind} = [S](t)$.

Maintaining the quasi-steady-state conditions for in vitro systems, we may assume constant association-dissociation rates so that probability of reaction is written as the rate of consumption of the substrate,

$$v(t) = -\frac{d}{dt}[S](t) \quad , \quad (8)$$

to saturation:

$$P_{\mathrm{REACT}}([S](t)) = \frac{v(t)}{V_{\max}} = -\frac{1}{V_{\max}}\frac{d}{dt}[S](t) \quad (9)$$

after the initial transient of the enzyme-substrate reaction in (1).

We know only that expression (9) behaves like a probability function, with values in the realm [0, 1]. Given expressions (2), (5) and (9) we derive an expression for the unreacted probability term, $P_{\mathrm{UNREACT}}([S](t))$.

The expression:

$$P_{\mathrm{UNREACT}}([S](t))^{\mathrm{MM}} = \frac{K_M}{[S](t) + K_M} \quad (10)$$

satisfies all of the probability requirements, including the limits in (7), and, when combined with equations (9) and (5), gives the instantaneous version of the classical Michaelis-Menten equation (2). Remarkably, expression (10) can be seeing as generalization of the efficiency of the Michaelis-Menten reaction under steady-state conditions [34]. Originally, the efficiency depends on two parameters: K_M that embodies the thermodynamic conditions of the enzymic reaction and the initial substrate concentration $[S_0]$; it determines the ratio of the free to total enzyme concentration in the reactions (1); that is, when the efficiency is equal to one, we cannot expect to find substrate free in the reaction, i.e. the reactions in (1) are all consumed so that first branch of the limits (7) is fulfilled as no further binding will occur.

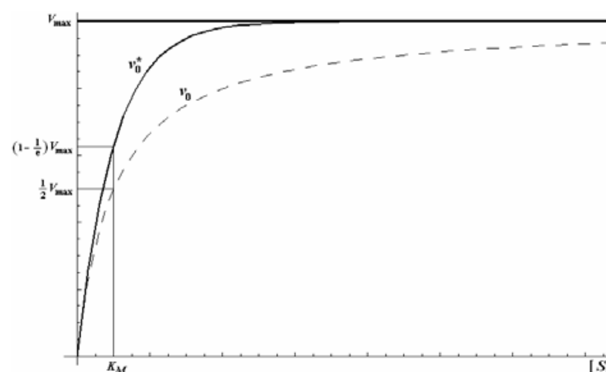


Fig. 1. Initial Michaelis-Menten and logistic velocities plotted against initial substrate concentration for the reaction (1). The dashed curve corresponds to the Michaelis-Menten equation (2) while the continuous thick curve represents its logistic generalization:

$$v_0^* = V_{\max}\left[1 - \exp\left(-[S_0]/K_M\right)\right]$$

It is clear that the Michaelis-Menten term (10) is just a particular choice for a probabilistic enzymatic kinetic model of the conservation law (5). A more generalized version of equation (10) that preserves all of the above probabilistic features is

$$P_{\mathrm{UNREACT}}([S](t))^{*} = e^{-\frac{[S](t)}{K_M}} \quad (11)$$

from which the Michaelis-Menten term (10) is returned by performing the $[S](t)$ first order expansion for the case where the bound substrate approaches zero:

$$P_{\mathrm{UNREACT}}([S](t))^{*} = \frac{1}{e^{\frac{[S](t)}{K_M}}} \overset{[S](t)\to 0}{\cong} \frac{1}{1+\frac{[S](t)}{K_M}} = P_{\mathrm{UNREACT}}([S](t))^{\mathrm{MM}} \quad . \tag{12}$$

Worth noting that there is no monotonically form between 0 and 1 other that that of equation (11) to reproduce basic Michaelis-Menten term (10) when approximated for small x = $[S](t)/K_M$. For instance, if one decides to use exp(-x^2) then the unreactive probability will give 1/(1+x^2) as the approximation for small x, definitely different of what expected in basic Michaelis-Menten treatment (10). This way, the physico-chemical meaning of equation (12) is that the Michaelis-Menten term (10) and its associated kinetics apply to fast enzymatic reactions, i.e. for fast consumption of $[S](t)$, which also explains the earlier relative success in applying linearization and graphical analysis to the initial velocity equation (2).

Use of equation (11) instead of (10) expands the range of reaction rates and provides a new kinetic equation, in the form of a logistic expression

$$-\frac{1}{V_{\max}}\frac{d}{dt}[S](t) = 1 - e^{-\frac{[S](t)}{K_M}} \tag{13}$$

based on probability and derived from equations and (5), (9), and (11).

At initial conditions, logistic equation (13) gives an initial velocity of reaction (v_0^*) that is uniformly higher than that calculated by Michaelis-Menten (2) at all initial concentrations of the substrate, except for the case where $[S_0] \to 0$, when both are zero, see Fig. 1.

Reliability tests of the logistic form of Michaelis-Menten kinetics (13) are reported below.

3. Reliability of the logistic enzyme kinetic

Quasi Steady-State Approximation Analysis

One of the fundamental assumptions made in deriving basic Michaelis-Menten kinetics, except in the initial so-called transient phase of the reaction, is the quasi steady state approximation of the [*ES*] concentration, i.e. the rate of synthesis of the *ES* complex must equal its rate of consumption until the substrate is nearly exhausted. It has been demonstrated that the QSSA is equivalent with the physiologically common condition that the substrate is in great excess over the enzyme, as firstly shown by Laidler [35]:

$$[S_0] >> [E_0]. \tag{14}$$

Let us investigate whether condition (14) may arise within the proposed probabilistic enzymatic kinetics and what consequences that has for applicability of the logistic treatment.

For reaction (1) to proceed with a high probability it is necessary that

$$P_{\mathrm{REACT}}([S]_{bind}) \to 1 \Leftrightarrow P_{\mathrm{UNREACT}}([S]_{bind}) \to 0 \tag{15}$$

or, the probability of the enzymatic reaction proceeding increases to one as the probability that the substrate will not bind with the enzyme approaches zero. Analytically, we use the limiting case (16) where reaction (1) proceeds.

$$P_{\mathrm{REACT}}([S](t)) = 1 \tag{16}$$

Then, by combining equation (16) with the general in vitro form (9), we derive the time dependent equation (17).

$$-\frac{1}{V_{\max}}\frac{d}{dt}[S](t) = 1 \tag{17}$$

Substituting $V_{\max} = k_2[E_0]$ and integrating produces the linear portion of the substrate depletion curve:

$$[S](t) = [S_0] - k_2[E_0]t \ . \tag{18}$$

The substrate condition $[S](t) >> 0$ corresponds to the binding case for which equation (16) is valid under the conditions given in expression (6). Applying this substrate condition to equation (18) during the rate limiting step when

$$t \cong \frac{1}{k_2} \tag{19}$$

ensures that almost all of the substrate is being transformed into product via reactions (1), resulting in the QSSA condition (14).

We have proved that the left side of the probabilistic equivalence (15) is valid for QSSA and we must do the same for the right side. The more closely $P_{\mathrm{UNREACT}}([S](t))$ approaches zero as $P_{\mathrm{REACT}}([S](t))$ approaches one, the better QSSA is obtained.

Recalling the two forms presented for the non-binding reactivity, the Michaelis-Menten in equation (10) and the logistic in equation (11), we can clearly see that the following hierarchy exists

$$P_{\mathrm{UNREACT}}([S](t))^{\mathrm{MM}} = \frac{1}{1+\frac{[S](t)}{K_M}} > \frac{1}{e^{\frac{[S](t)}{K_M}}} = P_{\mathrm{UNREACT}}([S](t))^{*} \tag{20}$$

regardless of the time at which they are compared. Therefore, because the logistic probability P_{UNREACT} is lower than the Michaelis-Menten at all times, QSSA is better satisfied using the logistic approach.

Full Time Course Analysis

Many biochemists use the velocity equations for kinetic parameter estimates despite the fact that the rates are difficult to determine experimentally. In practice either the substrate depletion or the product formation is measured as a function of time and the rates are calculated by differentiating the data, leading to an inexact analysis [13, 23]. Alternatively, the differential equations governing the biochemical reactions may be solved or approximated to obtain reactant concentration as function of time. This approach decreases the number of experimental assays by at least a factor of five, as proved by Schnell and Mendoza [14], because multiple experimental points may be collected for each single reaction.

Unfortunately, until now, the most general analytical time-dependent solution for reaction (1) used the closed form (4) that has many mathematical disadvantages. For example it can return multiple values for the same argument [36] or result in an infinitely iterated exponential function [24].

To test whether the logistic kinetic equation (13), which is a natural generalization of the Michaelis-Menten equation, may provide a workable analytical solution in an elementary form we first integrate the equation

$$\int_{[S_0]}^{[S](t)} \frac{d[S](t)}{\exp(-[S](t)/K_M)-1} = \int_0^t V_{\max} dt \qquad (21)$$

generating the new equation to be solved:

$$[S_0]-[S](t)+K_M \ln\left(e^{-\frac{[S_0]}{K_M}}-1\right)-K_M \ln\left(e^{-\frac{[S](t)}{K_M}}-1\right)=V_{\max}t \qquad (22)$$

Although apparently more complex than the previous version (3), equation (22) can be solved exactly. This can be demonstrated by substituting

$$\varphi([S](t))=\frac{[S](t)}{K_M} \qquad (23)$$

into (22) to get the simple equation:

$$-\varphi([S](t))-\ln\left(e^{-\varphi([S](t))}-1\right)=\psi(t) \qquad (24)$$

where we have also introduced the functional notation:

$$\psi(t)=\frac{1}{K_M}\left(V_{\max}t-[S_0]\right)-\ln\left(e^{-\frac{[S_0]}{K_M}}-1\right). \qquad (25)$$

Now the exact solution of equation (24) is a logistic expression:

$$\varphi([S](t))=\ln\left(1-e^{-\psi(t)}\right). \qquad (26)$$

Finally, substituting function (25) into expression (26) gives the logistic progress curve for substrate consumption in an analytically elementary form:

$$[S]_L(t)=K_M \ln\left(1+e^{-\frac{V_{\max}t}{K_M}}\left(e^{\frac{[S_0]}{K_M}}-1\right)\right). \qquad (27)$$

This time-dependent solution (27) substitutes an elementary logarithmic dependency for the *W*-Lambert function. It is remarkable that the solution of a generalized logistic kinetic version of the Michaelis-Menten instantaneous equation provides an analytically exact solution.

The cutting test is in the comparison of the progress curves generated by the *W*-Lambert (4) and logistic solutions (27) respectively. To do this, the following working formulas for the instantaneous complex $[ES](t)$, product $[P](t)$ and enzyme $[E](t)$ concentrations are employed in both the *W*-Lambert (4) and logistic (27) versions of the binding substrate concentration, $[S]_{W,L}$, according with Schnell and Mendoza [13]:

$$[ES]_{W,L}(t)=\frac{[E_0][S]_{W,L}(t)}{[S]_{W,L}(t)+K_M}\left\{1-\exp\left[-k_1 t\left([S_0]+K_M\right)\right]\right\}, \qquad (28a)$$

$$[P]_{W,L}(t)=[S_0]-[S]_{W,L}(t)-[ES]_{W,L}(t), \qquad (28b)$$

$$[E]_{W,L}(t)=[E_0]-[ES]_{W,L}(t), \qquad (28c)$$

$$s_{W,L}(t)=\frac{[S]_{W,L}(t)}{[S_0]}, e_{W,L}(t)=\frac{[E]_{W,L}(t)}{[E_0]}, es_{W,L}(t)=\frac{[ES]_{W,L}(t)}{[E_0]}, p_{W,L}(t)=\frac{[P]_{W,L}(t)}{[S_0]}. \qquad (29)$$

The transformation:

$$\tau=1-\frac{1}{\ln(t+\mathrm{e})} \qquad (30)$$

allows us to use scaled time for the abscissa so that an infinite time range can be mapped onto the interval [0,1].

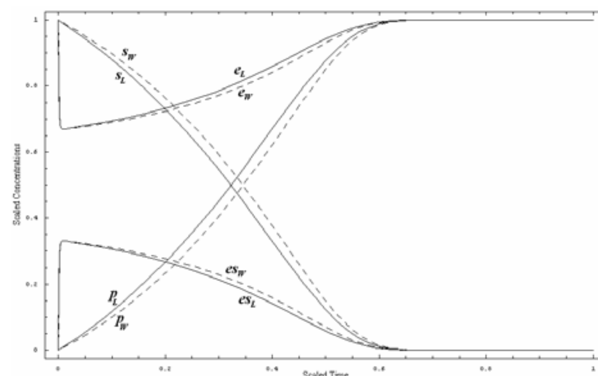


Fig. 2. Time dependent behavior of the reactant scaled concentrations (29) for the paradigmatic enzyme-substrate reaction (1) when the basic (dashed lines) and generalized logistic (solid lines) versions of the Michaelis-Menten kinetic are employed, with the parametric values $k_{-1}=k_2=10^2 s^{-1}$, $k_1=10^6 M^{-1}s^{-1}$, $[S_0]=10^{-4}M$, and $[E_0]=10^{-6}M$, against the scaled time (30).

Fig. 2 shows the plots of the *W*-Lambert and logistic progress curves (29) for an enzyme-catalyzed reaction in

vitro where $k_{-1}=k_2=10^2 s^{-1}$, $k_1=10^6 M^{-1}s^{-1}$, $[S_0]=10^{-4}M$, and $[E_0]=10^{-6}M$.

The quantitative behavior of the reactant concentrations in both the *W*-Lambert and logistic cases are strikingly similar. In addition, time-dependent product curves may be used instead of the initial velocity curves in Fig. 1. However, the logistic product curves are smoother and at higher concentrations than those obtained from the *W*-Lambert approach due to the higher probability of reaction (see the discussion from the previous section).

Having proved the reliability of the logistic time-dependent form of the substrate depletion expression (27) compared to the *W*-Lambert-based expression (4) we propose the general transformation [37]:

$$f_1 W\left(f_2 e^{f_2} e^{-f_3 t}\right) \to f_1 \ln\left(1+\left(e^{f_2}-1\right)e^{-f_3 t}\right), \quad (31)$$

where f_1, f_2, f_3 are factors that depend on K_M and $V_{\max}$, which is used to transform the closed form solutions of enzymatic kinetics into elementary analytical expressions. The particular relevance of the replacement (31) may be visualized from the Fig. 3.

As shown in Fig. 3, the difference in the shape of the curves generated by the general *W*-Lambert and natural logarithm functions (curve a) is almost completely removed when the *W*-Lambert time-dependent solution is replaced with the logistic one transformed as in (31) (curve b). This result suggests that using this logistic transformation (31) we get a good time-dependent representation over a broad range for enzymatic kinetics in vitro.

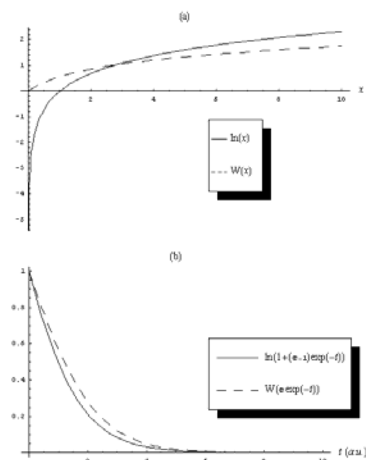


Fig. 3. a) Comparison of the W-Lambert function (dashed line) with the logarithmic function (solid line) against positive ranged simple arguments; b) Comparison of the W-Lambert function (dashed line) with logistic function (solid line) when the arguments include the temporal dependencies as in (31), respectively, being all involved factors fixed to unity. The time abscise scale in (b) is taken in arbitrary units.

This procedure can be directly applied to the existing *W*-Lambert type solutions for many enzymatic reactions in vitro, e.g. for enzyme inhibitors, for fully competitive enzyme reactions, for the enzyme kinetics of multiple alternative substrates [37] or for reversible enzyme kinetics [38], making them more useful for fitting laboratory data [39-41].

Analysis of Fitting Curves

Although they are able to use the progress curves for analysis of the data obtained from experimental assays, many biochemists prefer to use linear representations of enzyme kinetics. Instead of using the time-dependent solution (4), they rearrange the time-dependent equation (3) to a sort of time-dependent regression expression, for example, the reciprocal double plot equation:

$$\frac{t}{[S_0]-[S](t)} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}}\left(\frac{1}{[S_0]-[S](t)}\ln\frac{[S_0]}{[S](t)}\right). \quad (32)$$

A plot of equation (32) will yield a straight line with an intercept of $1/V_{\max}$ and a slope of $K_M/V_{\max}$ from which the kinetic parameters K_M and $V_{\max}$ can be obtained.

However, this approach has been criticized [40, 41] and it is worthwhile to investigate whether the exact logistic solution (27) may be better for fitting a linear curve.

First, we take advantage of the fact that the logistic solution (27) has an elementary form to take its derivative with respect to time. This provides an expression for the instantaneous velocity (8) which can be transformed to the finite difference $\left([S_0]-[S](t)\right)/t$.

Inversion of the result yields the expression:

$$\frac{t}{[S_0]-[S](t)} = \frac{1}{V_{\max}} + \frac{1}{V_{\max}\left(e^{\frac{[S_0]}{K_M}}-1\right)} e^{\frac{V_{\max}}{K_M}t}. \quad (33)$$

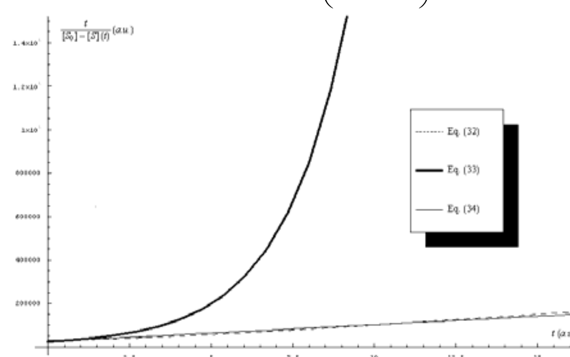


Fig. 4. Time dependent representation of the fitting curves (32)-(34) for the parametric values $k_{-1}=k_2=10^2 s^{-1}$, $k_1=10^6 M^{-1}s^{-1}$, $[S_0]=10^{-4}M$, and $[E_0]=10^{-6}M$. The dashed line corresponds to equation (32) and involves the W-Lambert closed form solution (4). The thin continuous line is the representation of linear equation (34) while the thick continuous line is the plot of non-linear equation (33) being both based on the logistic solution (27). Abscise and ordinate scales are given in arbitrary units.

Equation (33) is not a linear function, although it may be used for fitting the experimental time series data to determine the kinetic parameters K_M and $V_{\max}$. To obtain a linear equation from expression (33), recall that, from the probabilistic perspective of enzymatic kinetics, the Michaelis-Menten equation is valid for fast reactions. Performing a first order expansion with respect to time on (33) gives the linear equation:

$$\frac{t}{[S_0]-[S](t)} = \frac{e^{\frac{[S_0]}{K_M}}}{V_{\max}\left(e^{\frac{[S_0]}{K_M}}-1\right)} + \frac{1}{K_M\left(e^{\frac{[S_0]}{K_M}}-1\right)}t \quad (34)$$

Fig. 4 shows the comparison between linear fitting equation (32) and the new logistic based expression (34) along with the nonlinear form (33), for the same parameters used in Figure 2 above. Generation of the curve for expression (32) required that the *W*-Lambert time-dependence of the substrate depletion be substituted for the time dependent substrate concentration.

It is clear that the linear logistic curve (34) is nearly coincident with the Michaelis-Menten curve (32), both providing linear approximations of the general non-linear logistic curve (33). This is the first instance of treating substrate-enzyme binding probabilistically and it has the advantage of avoiding use of the *W*-Lambert function, which is impossible to evaluate exactly. The resulting linear fitting curves are essentially the same with either approach.

4. Conclusions

This work provides both a new interpretation and a new equation for Michaelis-Menten enzyme kinetics in vitro. We interpret the reaction between a substrate and an enzyme as a probabilistic process of physical-chemical binding. A set of constraints on the reactive and unreactive probabilities is also given. In this context the Michaelis-Menten unreactive term has the same form as the first order approximation of the more general logistic expression with respect to the degree of substrate-enzyme binding. The logistic version of the Michaelis-Menten equation and kinetics is thereby derived. The reliability of the logistic approach was tested by analyzing its ability to yield the quasi-steady-state approximation of enzyme-substrate synthesis and an analytical representation of the progress curves for the reactants as well as to provide the associated fitting equation for estimation of the kinetic parameters K_M and $V_{\max}$. In every case the logistic approach furnishes a better framework for characterizing and analyzing enzymic kinetics. It has also been proved that, in general, the Michaelis-Menten approach resembles a first order approximation of the time-dependent or substrate binding ranges and thus characterizes only fast enzymatic reactions.

From a mathematical perspective it is interesting to note that the more general probabilistic enzymatic kinetic problem is simpler than the classical Michaelis-Menten problem because the elementary solutions of the reactant progress curves and of the fitting equations is in better agreement with the quasi-steady-state condition. Although this probabilistic approach has been demonstrated theoretically, it would benefit from application to more complex experimental systems. The probabilistic approach in vitro may also be helpful in developing the stochastic or probability density-based biological theories needed to treat in vivo enzyme kinetics in the cell. Our effort to correlate in vitro and in vivo kinetics should strengthen the ability of biochemical kinetics to elucidate biomolecular functions, metabolism and the expression and transmission of genetic information.

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