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The condition for pseudo-first-order kinetics in enzymatic reactions is independent of the initial enzyme concentration

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Abstract

The linearization of the Michaelis–Menten reaction by pseudo-first-order kinetics is revised. A phase–plane analysis allows the derivation of a new condition for its validity that is directly linked to the reaction efficiency, and contrary to widely established knowledge, is independent of the initial enzyme concentration.

Keywords: Michaelis–Menten reaction; Pseudo-first-order kinetics; Flooding method; Progress curves; Rate constants; Fitting procedure

1. Introduction

The simplest reversible association between an enzyme E and a substrate S yields an intermediate enzyme–substrate complex C that irreversibly breaks down to form a product P:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \tag{1}$$

$$C \xrightarrow{k_2} E + P. \tag{2}$$

This reaction scheme is mathematically described by a set of coupled non-linear second-order differential equations that complicates the experimental determination of kinetic parameters. Such difficulties are largely solved in quasi-steady-state (QSS) conditions by measuring the initial rate of product formation as a function of initial substrate concentration, and then obtaining kinetic parameters by solving the Michaelis–Menten (MM) equation [1]:

$$v_0 = \frac{v_{\max}[S_0]}{K_M + [S_0]} \tag{3}$$

where $v_{\max}$ is the maximum velocity, $[S_0]$ is the initial substrate concentration and $K_M = (k_{-1} + k_2)/k_1$ is the MM constant. This equation is arguably among the most important in biochemistry.

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In the enzyme–substrate association (1), it is well-known that, if the initial enzyme concentration is much higher than the initial substrate concentration ($[E_0] \gg [S_0]$), the enzyme concentration $[E]$ remains effectively constant during the course of the reaction, and only the substrate concentration $[S]$ changes appreciably with time [2–6]. Since kinetic order with respect to time is the same as with respect to $[S]$, reaction (1) is said to follow pseudo-first-order (PFO) kinetics if the $[S]$ dependence is of first order. The rates of second-order reactions in chemistry are frequently studied within PFO kinetics [7,8]; in the present case, the second-order reaction $S + E \xrightarrow{k_1} C$ becomes mathematically equivalent to a first-order reaction reducing the MM mechanism (1)–(2) to:

$$S \underset{k_{-1}}{\overset{k_\psi}{\rightleftharpoons}} C \xrightarrow{k_2} P, \tag{4}$$

where $k_\psi \equiv k_1[E_0]$ is the pseudo rate constant. This liberalization procedure is also known as the method of flooding [9]. The solution of the governing equations for a reaction linearized by flooding is straightforward, and is widely employed to characterize kinetics and fit parameters with the aid of progress curves. However, as error is present due to the fact that the concentration of the excess reactant does not remain constant [7].

Numerical studies of reaction (1) far from the QSS or equilibrium approximations demonstrate that if the excess reactant concentration ratio, $[E_0]$:$[S_0]$ say, is less than 10-fold, appreciable errors are introduced in the PFO description [5]. Silicio and Peterson [7] have numerical estimates for second-order reactions that show that the fractional error in the observed PFO constant is less than 10% if the reactants ratio is 10-fold. However, Corbett [10] has found that a PFO reaction can yield more accurate data than is generally realized, even if only a two-fold excess of one the reactants is employed. For enzyme catalyzed reactions, Kasserra and Laidler [2] suggest that an excess of initial enzyme concentration is necessary to guarantee that the reaction follows first-order kinetics in transient-phase studies. Pettersson [4] introduces an additional assumption, namely that the amount of complex concentration accumulated during the short transient is too small to cause any significant kinetic effects on the enzyme or product concentration. These results appear to indicate that the conditions whereby a second-order enzymatic reaction is reduced to first order are not well established.

In a previous report, Schnell and Maini [11] have shown that, under the condition $[E_0] \gg [S_0]$, the appropriate framework to study the MM reaction (1)–(2) is the reverse quasi-steady-state approximation (rQSSA) or equilibrium approximation. The rQSSA considers the substrate S to be in a QSS with respect to the enzyme–substrate complex C by assuming that $\mathrm{d}[S]/\mathrm{d}t \approx 0$. From a biophysical point of view, S is in a QSS when $[E_0]$ is higher because all the molecules of the former readily combine with those of the latter. It also seems reasonable to state that, if $[E_0] \gg [S_0]$, the reaction is effectively of first order since the enzyme concentration is hardly affected during the reaction.

It is then widely believed that second-order reactions can be studied by PFO kinetics using progress curves only when the excess concentration of one of the reactants, in our case the enzyme, is large [8,9, for example]. By considering the reaction dynamics, it is shown here for the first time that this condition does not provide the general principle for the validity of the flooding method in enzyme catalyzed processes far from the QSS or the equilibrium approximations, and that a more general condition is independent of the enzyme concentration. In Section 2 the reduction of the MM reaction by PFO kinetics is summarized followed by its dynamical analysis (Section 3). The validity condition is derived in Section 4 and a brief discussion of its implications is given in Section 5.

2. The flooded Michaelis–Menten reaction

The time evolution of reactions (1)–(2) is obtained by applying the law of mass action to

yield the set of coupled non-linear differential equations:

$$\frac{d[S]}{dt} = k_1\Big(-\big([E_0]-[C]\big)[S]+K_S[C]\Big) \tag{5}$$

$$\frac{d[C]}{dt} = k_1\Big(\big([E_0]-[C]\big)[S]-K_M[C]\Big) \tag{6}$$

$$\frac{d[P]}{dt} = k_2[C] \tag{7}$$

and by imposing the conservation laws:

$$[E_0] = [E](t)+[C](t) \tag{8}$$

$$[S_0] = [S](t)+[C](t)+[P](t) \tag{9}$$

with initial conditions at $t=0$:

$$([S],[E],[C],[P]) = ([S_0],[E_0],0,0). \tag{10}$$

In this system the parameters k_1, k_{-1} and k_2 are positive rate constants, $K_S = k_{-1}/k_1$ is the equilibrium dissociation constant, $K = k_2/k_1$ the Van Slyke–Cullen constant and $K_M = K_S + K$ is known as the MM constant [11].

In the flooding method, the second-order enzyme–substrate interaction in reaction (1) is neglected when $[E_0] \gg [S_0]$; the reaction effectively becomes of first order since the concentration of E is hardly affected, that is:

$$[E_0]-[C] \approx [E_0]. \tag{11}$$

By substituting Eq. (11) in Eqs. (5)–(7), the system of equations is reduced to:

$$\frac{d[S]}{dt} = k_\psi\Big(-[S]+\tilde{K}_S[C]\Big) \tag{12}$$

$$\frac{d[C]}{dt} = k_\psi\Big([S]-\tilde{K}_M[C]\Big) \tag{13}$$

$$\frac{d[P]}{dt} = k_2[C] \tag{14}$$

where $k_\psi = k_1[E_0]$, $\tilde{K}_S = k_{-1}/k_\psi$, $\tilde{K} = k_2/k_\psi$ and $\tilde{K}_M = \tilde{K}_S + \tilde{K}$. Note that these equations can also be obtained by applying the law of mass action to reaction scheme (4). Solutions for Eqs. (12)–(14) with the prescribed conservation law (9) and the initial condition (10) can be obtained by direct integration [12] or by Laplace's transforms; for $k_\psi \neq k_{-1} \neq k_2$, they take the form:

$$[S](t) = \frac{[S_0]}{\lambda_1-\lambda_2}\big((\lambda_1-k_\psi)\exp(-\lambda_2 t) - (\lambda_2-k_\psi)\exp(-\lambda_1 t)\big) \tag{15}$$

$$[C](t) = [S_0]\frac{k_\psi}{\lambda_1-\lambda_2} \times(\exp(-\lambda_2 t)-\exp(-\lambda_1 t)) \tag{16}$$

$$[P][t] = [S_0]\left(1+\frac{\lambda_2}{\lambda_1-\lambda_2}\exp(-\lambda_1 t) - \frac{\lambda_1}{\lambda_1-\lambda_2}\exp(-\lambda_2 t)\right) \tag{17}$$

where

$$\lambda_1 = \frac{k_\psi}{2}\Big(\big(1+\tilde{K}_M\big)+\sqrt{\big(1+\tilde{K}_M\big)^2-4\tilde{K}}\Big) \tag{18}$$

$$\lambda_2 = \frac{k_\psi}{2}\Big(\big(1+\tilde{K}_M\big)-\sqrt{\big(1+\tilde{K}_M\big)^2-4\tilde{K}}\Big), \tag{19}$$

assuming by definition that $\lambda_1 > \lambda_2$. These progress curves obey biphasic kinetics [13], i.e. if the eigenvalues λ_1 and λ_2 differ significantly in magnitude ($\lambda_1 \gg \lambda_2$), there are then two timescales: a fast $(\lambda_1)^{-1}$ and a slow $(\lambda_2)^{-1}$. Further simplifications can be carried out if the initial transient is fast and cannot be measured [2,3,14].

Experimentally, the clear advantage of the flooding method when applied to the MM reaction is that, as shown by Eqs. (15)–(17), it provides closed form solutions whereby a single fit of the

substrate decay or progress curves can lead to its complete characterization, namely the three rate constants k_1, k_{-1} and k_2. If the initial enzyme concentration ($[E_0]$) is known, the rate constants can be determined from the kinetic data as follows:

$$k_1 = \frac{k_\psi}{[E_0]}, \quad k_{-1} = k_\psi(\tilde{K}_M - \tilde{K}), \quad k_2 = k_\psi \tilde{K}, \tag{20}$$

where

$$\tilde{K}_M = \frac{\lambda_1 + \lambda_2}{k_\psi} - 1 \quad \text{and} \quad \tilde{K} = \frac{\lambda_1 \lambda_2}{k_\psi^2}. \tag{21}$$

By contrast, complete characterizations based on QSS conditions require the progress fits of two reaction components [11,15,16]. Kineticists also measure individual rate constants employing sophisticated methods such rapid mixing, sampling techniques, flash photolysis and relaxation methods [6,17], but multiple measurements of the steady-state approach rate are required since it is during this period that the individual rate constants can be observed.

It has been previously assumed that the condition $[E_0] \gg [S_0]$ implies:

$$[E](t) = [E_0] - [C](t) \approx [E_0] \Rightarrow \left| \frac{[C]}{[E_0]} \right|_{\max} \ll 1. \tag{22}$$

From a biophysical point of view, it seems reasonable to overestimate the maximum complex concentration when there is an enzyme excess by assuming that all the substrate molecules instantaneously combine with the enzyme molecules, i.e. $[C]_{\max} = [S_0]$. In our view, this is an oversimplification as it is physically unrealistic to assume that in the conservation law (8) all the enzyme molecules are only in one form: the free enzyme (see, Eq. (22)). A more realistic condition for the validity of the flooded MM reaction (1)–(2), i.e. through a reliable estimate of $[C]_{\max}$, can be derived by a geometrical study of the phase plane of the system (5)–(6) which is carried out below.

3. Phase–plane analysis of the Michaelis–Menten reaction

As derived independently by Roussel and Fraser [18] and Schnell and Maini [11], the phase–plane curves of the differential equation system (5)–(6) are determined through the expression:

$$[C] = \frac{[E_0][S]}{\phi + [S]} \tag{23}$$

with

$$\phi = K_S + \frac{K}{1 + \mathrm{d}[C]/\mathrm{d}[S]}. \tag{24}$$

The substitution of Eq. (23) in Eq. (7) yields the general product formation velocity:

$$v = \frac{v_{\max}[S]}{\phi + [S]} \tag{25}$$

where $v_{\max} = k_2[E_0]$ is the maximum velocity. Under certain conditions, Eq. (25) can be used to derive simpler velocity relations to estimate the reaction parameters: $v_{\max}$, K_S and K. Two important facts emerge from Eqs. (23) and (24) [19].

1. For $[E_0] \ll K_M + [S]$, the standard quasi-steady-state approximation (sQSSA) can be applied: $\mathrm{d}[C]/\mathrm{d}t \approx 0$ and hence $\mathrm{d}[C]/\mathrm{d}[S] \to 0$ and $\phi = K_S + K = K_M$ [15,20]. This regime is usually referred to as Briggs–Haldane kinetics [6,21].
2. When $[E_0] \gg [S_0]$, the rQSSA holds: $\mathrm{d}[S]/\mathrm{d}t \approx 0$ and hence $\mathrm{d}[C]/\mathrm{d}[S] \to \infty$ and $\phi = K_S$ [11]. This approximation is known as Michaelis–Menten kinetics [6,22].

The phase plane is thus divided into three regions by the nullclines:

$$[C_r] = \frac{[E_0][S]}{K_S + [S]} \tag{26}$$

$$[C_s] = \frac{[E_0][S]}{K_M + [S]} \tag{27}$$

obtained by, respectively, setting $\mathrm{d}[C]/\mathrm{d}t \approx 0$ and $\mathrm{d}[S]/\mathrm{d}t \approx 0$ in Eq. (23). It may be noted that since $K_M > K_S$, then $[C_r] > [C_s]$ for $[S] = (0, \infty)$, and that

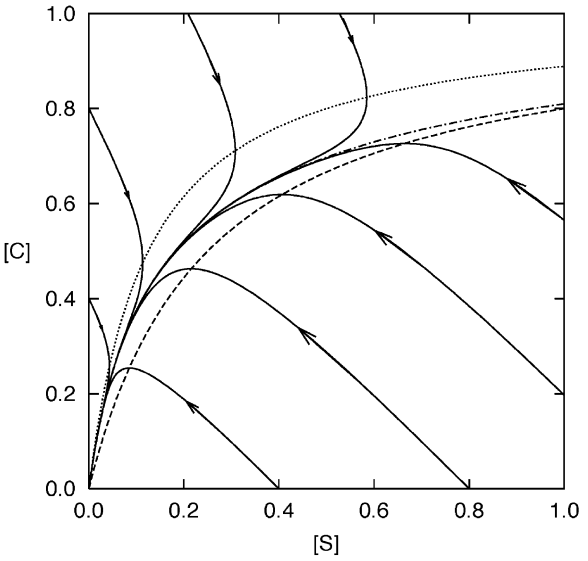

Fig. 1. Phase–plane behaviour of the enzyme–substrate reaction (1)–(2) obtained by the numerical solution of Eq. (23). Solid curves with arrows depict trajectories, the upper dotted curve being the rQSSA nullcline (26) and the lower dashed curve the sQSSA nullcline (27). The invariant manifold is given by the dotted–dashed curve and was plotted using the DEtools library in Maple. Parameter values are: $K=1/8$, $K_S=1/8$, $K_M=1/4$ and $[E_0]=1$.

the nullclines converge at large substrate concentrations because:

$$\lim_{[S]\to\infty}[C_s]=\lim_{[S]\to\infty}[C_r]=[E_0]. \tag{28}$$

The local behaviour can be studied by a linear stability analysis of system (5)–(6) at the equilibrium point $[S]=[C]=0$ which shows that solutions converge to a globally stable node. This occurs because the MM reaction is a closed and isothermal chemical system [23,24].

Phase–plane trajectories of expression (23) and its nullclines (26)–(27) are shown in Fig. 1. It may be seen that the trajectories cross the rQSSA nullcline (26) vertically and the sQSSA nullcline (27) horizontally. After the initial transient, the flow is attracted by the global invariant manifold confined between the two nullclines, and all the trajectories tend to the point (0,0) (chemical equilibrium) as $t\to\infty$. From these observations, it follows that the long-term behaviour of the MM reaction (1)–(2), namely the global invariant manifold of the phase plane, is bounded by:

$$0\le[C_s]\le[C]\big([S]\big)\le[C_r]<[E_0]\quad\text{for } t>t_Q. \tag{29}$$

This condition has been previously reported [25–27], and the convergence proofs of Segel and Slemrod [28] rigorously detail the position of the invariant manifold. Here condition (29) is now formally confirmed.

Following Crooke et al. [29], it can be demonstrated that, after an initial transient, any solution of (23) is bounded between the sQSSA ($\mathrm{d}[C]/\mathrm{d}[S]\to 0$) and the rQSSA ($\mathrm{d}[C]/\mathrm{d}[S]\to\infty$) nullclines by defining the QSS time, t_Q, as:

$$\left.\frac{\mathrm{d}C}{\mathrm{d}S}\right|_{t_Q}=0. \tag{30}$$

t_Q is the time at which biochemically realistic trajectories (i.e. those that start from the positive horizontal axis and obey initial condition 10) reach their $[C]$-maximum value in the positive $[S]$–$[C]$ phase plane. It can be seen in Eq. (23) that $[C]$ has a strict maximum within $(0,[S_0])$ by letting $[S_Q]$ be the concentration of the substrate at time $t=t_Q$ for which $\mathrm{d}[C]/\mathrm{d}[S]=0$. The concentration of the complex at $t=t_Q$ is given by setting $\mathrm{d}[C]/\mathrm{d}[S]=0$ in expression (23):

$$[C_Q]=\frac{[E_0][S_Q]}{K_M+[S_Q]}. \tag{31}$$

The second derivative of $[C]$ at $([S_Q],[C_Q])$ yields:

$$\left.\frac{\mathrm{d}^2[C]}{\mathrm{d}[S]^2}\right|_{([S_Q],[C_Q])}=-\frac{K_M}{K[S_Q]} \tag{32}$$

showing that $[C]$ reaches a maximum at the critical point $([S_Q],[C_Q])$ which lies on the sQSSA nullcline (27). Furthermore, since the sQSSA nullcline $[C_S]$ is monotonically increasing and $[C]$ cannot have an inflection point, $([S_Q],[C_Q])$ is unique on the positive $[S]$–$[C]$ phase plane. This analysis shows that $[C_S]<[C]$ for $t>t_Q$. Moreover, it can

be seen that the curves $[C_r]$ and $[C]$ can only intersect within $(0,[S_0])$ if $d[C]/d[S] \to \infty$ which is impossible for trajectories departing from the horizontal axis. Hence, it can be concluded that $[C_r] > [C]$ for $t > t_Q$.

These results can be further corroborated by analysing the bounds of the product formation velocity (7) [30]. By solving (6) for $[C]$ and substituting in (7), we obtain:

$$\frac{d[P]}{dt} = \frac{v_{max}[S]}{K_M + [S]} - \frac{K}{K_M + [S]} \frac{d[C]}{dt}. \tag{33}$$

In the QSS regime, $d[C]/dt < 0$ thus:

$$\frac{d[P]}{dt} \geq \frac{v_{max}[S]}{K_M + [S]} \tag{34}$$

which implies that the lower bound for the product formation velocity is that of the sQSSA. To obtain the upper bound, we take $d[C]/dt$ from the conservation law (9):

$$\frac{d[C]}{dt} = -\left(\frac{d[S]}{dt} + \frac{d[P]}{dt} \right) \tag{35}$$

and replace it in Eq. (33):

$$\frac{d[P]}{dt} = \frac{v_{max}[S]}{K_S + [S]} + \frac{K}{K_S + [S]} \frac{d[S]}{dt}. \tag{36}$$

As $[S]$ always decreases $(d[S]/dt < 0)$, the upper bound for the velocity of product formation is:

$$\frac{d[P]}{dt} \geq \frac{v_{max}[S]}{K_S + [S]}, \tag{37}$$

i.e. the rQSSA product formation velocity. Therefore, the velocity of product formation is bounded by:

$$\frac{v_{max}[S]}{K_M + [S]} \leq \frac{d[P]}{dt} \leq \frac{v_{max}[S]}{K_S + [S]} \tag{38}$$

which reveals an important property of the MM reaction (1)–(2): after the initial transient, trajectories enter a region trapped between the sQSSA and the rQSSA and rapidly converge to the global invariant manifold. Moreover, from the phase–plane deductions outlined above, it can be concluded that the trajectories are convex and reach a maximum at the intersection with the sQSSA nullcline (see Fig. 1).

4. Validity of PFO kinetics

In the analysis in Section 3, it has been demonstrated that the maximum value of $[C]_{max} = [C_Q]$ is given by expression (31). By substituting the latter into Eq. (22), the condition for PFO kinetics (flooding) to be valid can be expressed as:

$$\left| \frac{[C]}{[E_0]} \right|_{max} = \frac{[S_Q]}{K_M + [S_Q]} \ll 1. \tag{39}$$

Fig. 2 shows phase–plane solutions of Eq. (23) with the initial conditions $s(0) = 1$, $c(0) = 0$, $k_1 = 1.1$, $k_{-1} = 1$ and $k_2 = 0.1$. Note that the substrate (s) and complex (c) dimensionless variables are, respectively, defined with the $[S_0]$ and $[E_0][S_0]/(K_M + [S_0])$ scales. Thus, s decreases from an initial unitary value while c rapidly attains its maximum value of order unity and then reduces to zero. Segel [20] has previously shown that the complex concentration reaches its maximum value and the substrate concentration does not decrease appreciably from its initial concentration when:

$$\sigma \equiv \frac{[E_0]}{K_M + [S_0]} \ll 1. \tag{40}$$

Fig. 2 also shows that for $\sigma = 0.01$ and $\sigma = 0.1$ there are very small decreases in substrate concentration during the initial transient whereas c rises close to unity, that is $[S_0] \approx [S_Q]$. When σ is unity, expression (39) is less than (40), as expected, as there is a significant decrease of substrate concentration during the initial transient. Nonetheless, $[S_Q]$ is always bounded by $[S_0]$. Since $[S_Q] \leq [S_0]$ (see Fig. 2), condition (39) is always obeyed within the more general constraint:

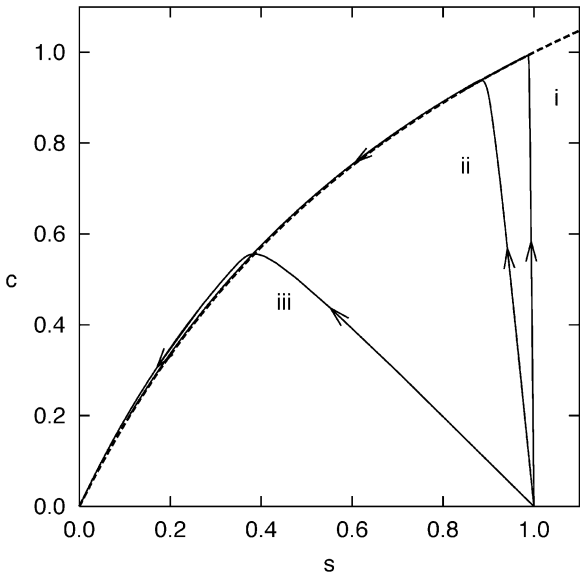

Fig. 2. Numerical solutions of expression (23) describing the phase plane of the single enzyme–substrate reaction (1)–(2). The initial conditions are $s(0)=1$, $c(0)=0$, $k_1=1.1$, $k_{-1}=1$ and $k_2=0.1$. (i) $[E_0]=2$ ($\sigma=0.01$); (ii) $[E_0]=0.2$ ($\sigma=0.1$) and (iii) $[E_0]=0.02$ ($\sigma=1$). Note that the trajectories rapidly reach the sQSSA nullcline (dashed curve) for cases (i) and (ii). There is also little substrate depletion during the initial transient. However, the substrate decays appreciably during the initial transient for case (iii).

$$\left|\frac{[C]}{[E_0]}\right|_{\max} \leq \frac{[S_0]}{K_M+[S_0]} \ll 1 \tag{41}$$

which can then be taken as the formal validity condition. The interesting feature of this new condition for flooding is that it is independent of the enzyme concentration and that it can be associated to the MM reaction efficiency:

$$\varepsilon \equiv \frac{K_M}{K_M+[S_0]} \tag{42}$$

as derived by Schnell and Mendoza [31,32] and to the elasticity of the MM reaction [33,34]; that is, when PFO kinetics is a valid approximation the reaction efficiency is $\varepsilon \approx 1$. The efficiency determines the ratio of the free to total enzyme concentrations in the reaction. It depends on two parameters: the MM constant K_M that embodies the thermodynamic environment of the reaction and the initial substrate concentration. Therefore, condition (41) states that the proportion of occupied enzyme is small during the reaction when the PFO kinetics is valid, that is when $[S_0] \ll K_M$ which is independent of the initial enzyme concentration.

Condition (41) has an important consequence. The commonly quoted condition for the flooded MM reaction ($[E_0] \gg [S_0]$) is neither sufficient nor necessary. For instance, when $[E_0]=[S_0]$ the MM reaction can still be reduced to PFO kinetics provided $[S_0] \ll K_M$. This is illustrated in Fig. 3 where the percentage error introduced by flooding with respect to the exact numerical solution is plotted for different $[S_0]:K_M$ ratios with the initial conditions $[S_0]=[E_0]=1$ and $k_1=2$. This error is approximately 20% for $[S_0]:K_M=1/3$, 10% for $[S_0]:K_M=1/10$ and less than 1% for $[S_0]:K_M=1/100$. We would like to emphasize that our numerical calculations confirm previous results: the maximum error decreases with the $[S_0]:[E_0]$ ratio. However, we also find that the FPO kinetics is a good approximation for $[E_0] \gg [S_0]$ provided that $[S_0] \ll K_M$. When $[S_0]:[E_0]=1/10$ the maximum percentage error is about of 24% for $[S_0]:[K_M]=10$. The maximum percentage error also diminishes with the $[S_0]:[K_M]$ ratio. This clearly demonstrates that the commonly quoted conditions for PKO kinetics in the MM reaction are incorrect, and that our new condition (41) is in fact sufficient.

5. Discussion

In the present work, we have investigated the application of the PFO approximation or flooding method to the MM reaction. PFO kinetics serves the purpose of linearizing the set of differential equations governing the time course of the reaction, which can be validated by a proper choice of conditions. In the past, it has been stated that the only necessary and sufficient condition for the application of PFO kinetics to the MM reaction far from the QSS and equilibrium approximations is $[E_0] \gg [S_0]$ [2–6]. By considering the reaction dynamics, it is shown here for the first time that this condition does not provide the general principle for the validity of the flooding method in

enzyme catalyzed processes, and is in fact incorrect. We have derived a more general expression independent of the enzyme concentration, namely $[S_0] \ll K_M$, and have showed that this is the sufficient condition. Furthermore, it is valid in a broader parameter domain as percentage errors introduced have been shown to be less than 1% for initial enzyme and substrate equimolar conditions. We are currently exploring the differences between the validity conditions for PFO kinetics in catalyzed and non-catalyzed reactions which will be reported elsewhere.

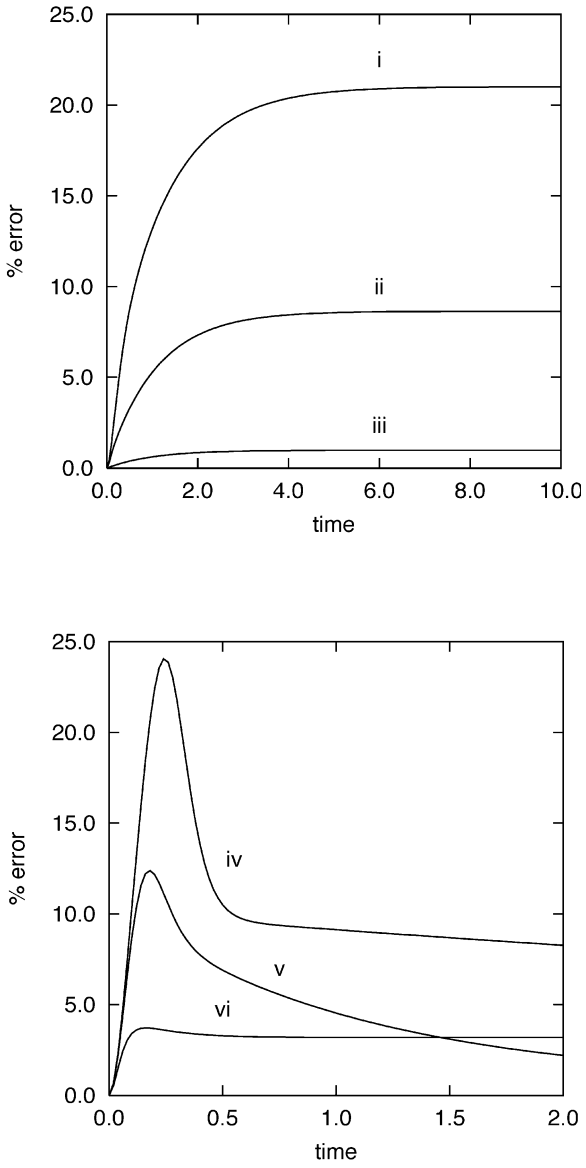

Fig. 3.

The PFO approximation leads to analytical solutions of the kinetic differential equation in the explicit form expressed by Eqs. (15)–(17). Experimentally, the clear advantage of these solutions is that, if the initial enzyme concentration is known, a single fit of the substrate decay leads to the determination of the three rate constants k_1, k_{-1} and k_2. It also provides a general kinetic method for investigating the active site of enzyme and enzyme-like catalysts because it facilitates the measurement of the first-order rate constant k_2 or the turn-over number [6].

Transient-state kinetics are becoming invaluable in the measurement of the rate constants of enzymatic reactions in the postgenomic era due to an increase in the studies of mutant and molecular engineering proteins. Although comparisons between the kinetic properties of wild-type and mutant enzymes have been long made in the past, it has been difficult to reach kinetic conclusions because the prevailing methodologies have not been very accurate [19]. This view is changing with the recent development of sophisticated methods such as rapid mixing, sampling techniques, flash photolysis and relaxation methods [6,17]. However, one of the major problems of the transient kinetic behaviour of enzymatic systems far from the QSS or equilibrium approximations is that it is fairly involved, and closed form solutions of progress curves can be derived only for a few mechanisms [35]. The MM reaction is not an exception, but PFO kinetics will facilitate its transient-state studies.

Fig. 3. Percentage error introduced by the PFO approximation with respect to the exact numerical solution for different $[S_0]$:$[K_M]$ ratios. The initial conditions are taken to be $[S_0]=1$, $k_1=2$. The percentage error is plotted for equimolar condition, $[S_0]=[E_0]=1$, in curves (i)–(iii). The ratios are as follows: (i) $[S_0]$:$K_M=1/3$ ($k_{-1}=k_2=3$); (ii) $[S_0]$:$K_M=1/10$ ($k_{-1}=k_2=10$) and (iii) $[S_0]$:$K_M=1/100$ ($k_{-1}=k_2=100$). The percentage error for $[S_0]$:$[E_0]=1/10$ is shown in curves (iv)–(vi). The $[S_0]$:K_M ratios are (iv) $[S_0]$:$K_M=10$ ($k_{-1}=k_2=0.1$); (v) $[S_0]$:$K_M=1$ ($k_{-1}=k_2=1$) and (vi) $[S_0]$:$K_M=1/10$ ($k_{-1}=k_2=10$).

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