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Solution to Michaelis–Menten enzyme kinetic equation via undetermined gauge functions: Resolving the nonlinearity of Lineweaver–Burk plot

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A composite approximate solution of Michaelis–Menten enzyme kinetic equation, which could describe both transient and slow dynamics, was obtained by ordinary perturbation methods in terms of undetermined gauge functions up to a first-order level. It was found that the zeroth-order perturbation function itself solved the paradox due to steady-state approximation and predicted well the maximum enzyme-substrate complex ($[ES]_{max}$) and time t_m to attain it. Extensive kinetic simulations using a chemical kinetic simulator proved the validity of these results. A comparison between simulated and predicted results showed that error in the prediction of t_m was negligible when perturbation parameter falls in the range of ($0 < \varepsilon \ll 1$). Apart from these, also the effect of transient dynamics on the linearity of Lineweaver–Burk plot (especially near the origin) has been explained. © *2002 American Institute of Physics.* [DOI: 10.1063/1.1496459]

INTRODUCTION

Kinetics of enzyme reactions is very important in biology, as well as in chemistry. The simplest enzyme kinetic model which is most well known Michaelis–Menten[1] model, can be described by

$$E + S \underset{k_2}{\overset{k_1}{\leftrightarrow}} ES \overset{k_3}{\rightarrow} E + P,$$
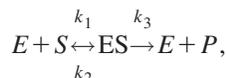
where E, S, ES, and P represent enzyme, substrate, enzyme–substrate complex, and products, respectively. Here k_1 (mol^{-1} s^{-1}), k_2 (s^{-1}) and k_3 (s^{-1}) are the respective rate constants. The system of coupled differential equations for this model can be given as

$$\frac{d[ES]}{dt} = k_1([E_0] - [ES])([S_0] - [ES] - [P])$$
$$- (k_2 + k_3)[ES]$$
$$\frac{d[E]}{dt} = -\frac{d[ES]}{dt} = \left(\frac{d[S]}{dt} + \frac{d[P]}{dt}\right) \quad (1)$$
$$\frac{d[P]}{dt} = k_3[ES].$$

Since finding an explicit analytical solution for system (1) is impossible, usually this will be approximated by either steady-state (SSA) or equilibrium methods.[2–4] The SSA is based on the assumption that,

$$\lim_{t \to \infty} \frac{d[ES]}{dt} \approx 0, \quad (2)$$

and it is valid only when the following inequalities are true.

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$$[E_0] \ll [S_0], \quad k_2 \gg k_3, \quad [S_0] \approx [S], \quad [ES] \ll [E_0]. \quad (3)$$

The necessity of aforementioned condition can be proved as follows: Under condition (3), system (1) can be approximated to

$$\frac{d[ES]}{dt} = k_1([E_0] - [ES])[S] - (k_2 + k_3)[ES]. \quad (4)$$

The solution to Eq. (4) for the initial condition $[ES]_{t=0} = 0$ can be given as

$$[ES] = \frac{[E_0][S]}{[S] + \left(\dfrac{k_2 + k_3}{k_1}\right)}(1 - e^{-(k_1[S] + k_2 + k_3)t}), \quad (5)$$

and its derivative becomes

$$\frac{d[ES]}{dt} = k_1[E_0][S]e^{-(k_1[S] + k_2 + k_3)t}. \quad (6)$$

Now it is easy to verify that Eq. (6) satisfies SSA conditions given by Eq. (2) as

$$\lim_{\substack{[S] \to \infty \\ t \to \infty}} \left(\frac{d[ES]}{dt}\right) = 0. \quad (7)$$

Therefore, the corresponding SSA limits are

$$\lim_{t \to \infty} [ES] = \frac{[E_0][S]}{[S] + \left(\dfrac{k_2 + k_3}{k_1}\right)},$$

$$\lim_{t \to \infty} v = \frac{d[P]}{dt} = k_3[ES] = \frac{V_{max}[S]}{[S] + K_m}, \quad (8)$$

where

$$V_{\max}=k_3[E_0], \quad K_m=\frac{k_2+k_3}{k_1}.$$

Here v is the reaction velocity and $V_{\max}$ is its maximum. Condition (3) may hold *in vitro* (under laboratory conditions) but definitely not *in vivo*[5–8] (inside the living cells) where the concentration of enzyme is always comparable with substrate concentration. Another paradox due to SSA is as follows: For system (1), the following inequality also should hold

$$[P]_\infty=\lim_{t\to\infty}\left(k_3\int_0^t[ES]dt\right)\leqslant[S_0], \tag{9}$$

which warns us against SSA given by Eq. (5) in that situation, $[P]_\infty$ will become infinite which is impossible. Since $[S]$ is evolving with time, the self-consistency [given by third inequality in Eq. (3)] of SSA will fail after certain period. Also, a problem due to bifurcation significantly modifies the SSA, limit[8] with respect to a slight change in the initial values.[9] Thus, in order to circumvent these disadvantages of SSA many attempts were made to solve system (1) by ordinary and singular perturbation methods in terms of inner (for the fast component) and outer (for the slow component) solutions.[10–12] In some attempts, the outer solutions of the singular perturbation series were expanded in terms of complicated Lambert's functions.[13,14] Recently, the transient and slow kinetics were treated using a double perturbation series, which yielded a partial decoupling at finite series expansion and complete decoupling at infinite series expansion.[3] Since most of the treatments simplified the problem by decoupling, they could not describe the dynamics for full time scale ($0\leqslant t\leqslant\infty$) using a single perturbation series. In order to combine the inner and outer solutions (to get the composite approximation), one has to use methods like the asymptotic matching principle of Van Dyke.[15] Since such solutions obtained from earlier approaches were complicated and cumbersome to apply, there was a need to develop a simple solution which could describe the fast (presteady state), slow (steady state), as well as the boundary layer dynamics better than SSA. Thus, the aim of this article is to develop a composite approximation to system (1) (which is practically applicable) by ordinary perturbation methods in terms of undetermined gauge functions, check its validity by stochastic simulations, and show the effect of transient dynamics on the linearity of the Lineweaver–Burk (LB) plot.

REDUCTION OF SYSTEM (1) TO PERTURBATION PROBLEM BY NEW SCALING SCHEME

System (1) can be written symbolically as,

$$\frac{dx}{dt}=k_1(e_0-x)(s_0-x-p)-(k_2+k_3)x, \tag{10}$$

$$\frac{dp}{dt}=k_3x, \tag{11}$$

where $[ES]=x$, $[S_0]=s_0$, $[E_0]=e_0$ and $[P]=p$. From Eq. (11),

$$x=\frac{1}{k_3}\left(\frac{dp}{dt}\right). \tag{12}$$

Using relation (12), Eq. (10) can be simplified to

$$\frac{d^2p}{dt^2}+(k_1(e_0+s_0)+k_2+k_3)\frac{dp}{dt}-k_1k_3e_0s_0$$
$$=-\left(k_1p+\frac{k_1}{k_3}\frac{dp}{dt}\right)\frac{dp}{dt}-k_1k_3e_0p. \tag{13}$$

The dimensionality of Eq. (13) can be removed by the following scheme of scaling

$$P=\frac{k_1s_0}{k_3e_0}p, \tag{14}$$

$$\tau=k_3t. \tag{15}$$

Therefore, Eq. (13) is reduced to the following perturbation problem with an ordinary perturbation parameter $\varepsilon=e_0/s_0$

$$\frac{d^2p}{d\tau^2}+\alpha\frac{dP}{d\tau}+\beta P-\gamma=-\varepsilon\left(\left(P+\frac{dP}{d\tau}\right)\frac{dP}{d\tau}\right), \tag{16}$$

where

$$\alpha=\frac{k_1(e_0+s_0)+k_2+k_3}{k_3}, \quad \beta=\frac{k_1e_0}{k_3},$$

$$\text{and} \quad \gamma=\left(\frac{k_1s_0}{k_3}\right)^2.$$

The perturbation parameter ε has the following limit when the SSA conditions hold

$$\lim_{\substack{s_0\to\infty\\s_0\gg e_0}}(\varepsilon)=0. \tag{17}$$

In the limit given by Eq. (17), Eq. (16) becomes an ordinary second-order linear differential equation with constant coefficients!

SOLUTION TO EQ. (13) BY UNDETERMINED GAUGE FUNCTIONS

The solution to Eq. (16) can be expanded in terms of gauge functions[16] as

$$P=P_0+\varepsilon P_1+o(\varepsilon^2). \tag{18}$$

Putting Eq. (18) into Eq. (16) and equating coefficients of similar powers of ε on left- and right-hand side, we obtain

$$\frac{d^2P_0}{d\tau^2}+\alpha\frac{dP_0}{d\tau}+\beta P_0-\gamma=0, \tag{19}$$

$$\frac{d^2P_1}{d\tau^2}+\alpha\frac{dP_1}{d\tau}+\beta P_1=-\frac{dP_0}{d\tau}\left(\frac{dP_0}{d\tau}+P_0\right), \tag{20}$$

$$\frac{d^2P_2}{d\tau^2}+\alpha\frac{dP_2}{d\tau}+\beta P_2=-\frac{dP_1}{d\tau}\left(\frac{dP_1}{d\tau}+P_1\right). \tag{21}$$

The gauge functions should satisfy the following initial conditions

$$|P_k|_{\tau=0}=0$$

$$\left|\frac{dP_k}{d\tau}\right|_{\rho=0}=0 \tag{22}$$

$$k=0,1,2.$$

The zeroth-order gauge function P_0 from Eq. (19) with the aforementioned initial conditions can be given as

$$P_0=\frac{\gamma}{\beta}+\frac{\gamma}{\beta(\delta_1-\delta_2)}(\delta_2 e^{\delta_1\tau}-\delta_1 e^{\delta_2\tau}), \tag{23}$$

where $\delta_{1,2}=\dfrac{-\alpha\pm\sqrt{\alpha^2-4\beta}}{2}$.

Also one should note that

$$(\delta_1+\delta_2)=-\alpha, \delta_1\delta_2=\beta, \tag{24}$$

and its first-order derivative can be given as

$$\frac{dP_0}{d\tau}=\frac{\gamma\delta_1\delta_2}{\beta(\delta_1-\delta_2)}(e^{\delta_1\tau}-e^{\delta_2\tau}) \tag{25}$$

putting

$$\delta_1=-u+v, \quad \delta_2=-u-v, \tag{26}$$

where $u=\dfrac{\alpha}{2}, \quad v=\dfrac{\sqrt{\alpha^2-4\beta}}{2}$.

Equations (23) and (25) can also be written as

$$P_0=\frac{\gamma}{\beta}-\frac{\gamma}{\beta\sinh(\eta)}e^{-u\tau}\sinh(v\tau+\eta), \tag{27}$$

$$\frac{dP_0}{d\tau}=\left(\frac{2\gamma}{v}\right)e^{-u\tau}\sinh(v\tau), \tag{28}$$

where $\eta=\tanh^{-1}\left(\dfrac{v}{u}\right)$.

Converting Eq. (23) and (25) to the original variables using relations (14) and (15)

$$[P]=p_0=s_0+\frac{s_0}{\sqrt{\alpha^2-4\beta}}(\delta_2 e^{\delta_1 k_3 t}-\delta_1 e^{\delta_2 k_3 t}), \tag{29}$$

$$[ES]=x=\frac{1}{k_3}\frac{dp_0}{dt}=\frac{k_1 s_0 e_0}{\sqrt{\alpha^2-4\beta}}(e^{\delta_1 k_3 t}-e^{\delta_2 k_3 t}). \tag{30}$$

This also can be written as in the form of Eq. (27) and (28) as

$$p=s_0-\frac{s_0}{\sinh(\eta)}e^{-k_3 u t}\sinh(k_3 v t+\eta), \tag{31}$$

$$[ES]=x=\frac{2k_1 e_0 s_0}{k_3\sqrt{\alpha^2-4\beta}}e^{-k_3 u t}\sinh(k_3 v t). \tag{32}$$

It is clear that Eq. (30) is a composite approximation (it contains the information of both transient and slow dynamics) of system (1), which satisfies the condition given by Eq. (9) and thus [ES] cannot be correctly approximated by SSA. When $(\delta_1 k_3)\cong 0$, we can see that Eq. (30) is reduced to SSA

and thus the first exponential is responsible for the slow dynamics. This is achieved because we did not decouple the system (1) completely. So the infinite expansion of series (18) will actually yield the exact solution!

NONLINEARITY IN LINEWEAVER–BURK DOUBLE RECIPROCAL PLOT

The nonlinearity of the usual set of Eadie–Hofstee plots has been discussed in earlier works.[17] Recently, based on the KT theory of Yang *et al.*,[18] the nonlinearity in the LB plot due to diffusive dynamics[19] has been explained. (Here the presteady-state rate constants are time dependent.) The effect of transient dynamics on the linearity of the LB plot can be shown as follows. When condition (3) holds, Eq. (30) reduces to SSA as

$$\delta_1\approx 0, \quad e_0\ll s_0$$

$$[ES]=x\approx\frac{k_1 s_0 e_0}{k_1 s_0+k_2+k_3}(1-e^{-(k_1 s_0+k_2+k_3)t_c}) \tag{33}$$

$$v=k_3 x=\frac{V_{max}s_0}{s_0+K_m}\varphi(s_0,t_c),$$

$$\varphi(s_0,t_c)=1-e^{-(k_1 s_0+k_2+k_3)t_c}. \tag{34}$$

Here, t_c is the reaction time, which is usually kept constant in experiments. Therefore, the equation for the LB plot becomes

$$\frac{1}{v}=\left(\frac{1}{V_{max}}+\frac{K_m}{V_{max}s_0}\right)\frac{1}{\varphi(s_0,t_c)}$$

$$=\left(\frac{1}{V_{max}}+\frac{K_m}{V_{max}}\left(\frac{1}{s_0}\right)\right)\left(\frac{1}{1-e^{-\left(\frac{k_1}{1/s_0}+k_2+k_3\right)t_c}}\right). \tag{35}$$

This clearly indicates that the LB plot is not linear when we keep reaction time t_c constant and change the substrate concentration. But this nonlinearity will dominate only when (k_2+k_3) or t_c is very small compared to k_1, especially near the origin. On the other hand, when these aforementioned factors are sufficiently large, then it is easy to verify that the nonlinear part in Eq.(35) becomes unity as in Eq.(36) and also this warns us about the datapoints to be chosen to calculate K_m values. So, wherever possible, only data points near the origin have to be taken for extrapolation and not data points away from the origin as they lead to an erroneous estimate of K_m and V_{max}. When we consider data points near the origin, it is easy to verify that the nonlinear term in Eq. (35) becomes unity as in Eq. (37)

$$\lim_{\substack{t_c\to\infty\\(k_2+k_3)\to\infty}}=\left(\frac{1}{\varphi(s_0,t_c)}\right)=1, \tag{36}$$

$$\lim_{\left(\frac{1}{s_0}\right)\to 0}\left(\frac{1}{\varphi(s_0,t_c)}\right)=1. \tag{37}$$

FIRST-ORDER PERTURBATION CORRECTION

The following results were generated using MAPLE7 mathematics software. Putting Eq. (27) in Eq. (20) and solving for P_1, which is the first-order gauge function, we obtain

$$\frac{dP_0}{d\tau}\left(\frac{dP_0}{d\tau}+P_0\right)=\frac{\gamma^2\delta_1\delta_2}{\beta^2}\left(\frac{1}{\delta_1-\delta_2}(e^{\delta_1\tau}-e^{\delta_2\tau})\right)+\frac{\gamma^2\delta_1\delta_2}{\beta^2}(\delta_2(1+\delta_1))e^{2\delta_1\tau}$$

$$+(\delta_1(1+\delta_2))e^{2\delta_2\tau}-((\delta_1+\delta_2)+\delta_1\delta_2)e^{(\delta_t\delta_2)\tau}),$$

$$(38)$$

$$\frac{d^2P_1}{d\tau^2}-(\delta_1+\delta_2)\frac{dP_1}{d\tau}+\delta_1\delta_2 P_1=\frac{\gamma^2\delta_1\delta_2}{\beta^2}\left(\frac{1}{\delta_1-\delta_2}(e^{\delta_1\tau}-e^{\delta_2\tau})\right)+\frac{\gamma^2\delta_1\delta_2}{\beta^2}(\delta_2(1+\delta_1))e^{2\delta_1\tau}$$

$$+(\delta_1(1+\delta_2+e^{2\delta_2\tau}-((\delta_1+\delta_2)+\delta_1\delta_2)e^{(\delta_1+\delta_2)\tau}).$$

$$(39)$$

The solution to Eq. (39) with an initial condition given by Eq. (22) can be given as

$$P_1=\frac{1}{\delta_1-\delta_2}\left(\left((n\delta_2-m)+\frac{\gamma^2((\delta_1-\delta_2)\tau+1)}{\beta(\delta_1-\delta_2)^2}\right)e^{\delta_1\tau}-\left((n\delta_1-m)+\frac{\gamma^2((\delta_1-\delta_2)\tau-1)}{\beta(\delta_1-\delta_2)^2}\right)e^{\delta_2\tau}\right)$$

$$+\frac{\gamma^2((2\delta_2+1)\delta_1+\delta_2)}{\beta^2(\delta_1-\delta_2)^2}e^{(\delta_1+\delta_2)\tau}\frac{\gamma^2}{\beta^2(\delta_1-\delta_2)^2}\left(\frac{\delta_1^2(1+\delta_2)}{2\delta_2-\delta_1}e^{2\delta_2\tau}+\frac{\delta_2^2(1+\delta_1)}{2\delta_1-\delta_2}e^{2\delta_1\tau}\right),$$

$$(40)$$

where

$$m=-\left(\frac{2\delta_1\delta_2\gamma^2}{\beta^2(\delta_1-\delta_2)^2}\left(\frac{\delta_1(1+\delta_2)}{2\delta_2-\delta_1}+\frac{\delta_2(1+\delta_1)}{2\delta_1-\delta_2}\right)\right.$$

$$\left.-\frac{\gamma^2((2\delta_2+1)\delta_1+\delta_2)(\delta_1+\delta_2)}{\beta^2(\delta_1-\delta_2)^2}\right),$$

$$n=\frac{\gamma^2}{\beta^2(\delta_1-\delta_2)^2}\left(((2\delta_2+1)\delta_1+\delta_2)\right.$$

$$\left.-\left(\frac{\delta_1^2(1+\delta_2)}{2\delta_2-\delta_1}+\frac{\delta_2^2(1+\delta_1)}{2\delta_1-\delta_2}\right)\right).$$

After putting P_0 and P_1 in Eq. (18), we obtain

$$P=P_0+\varepsilon P_1+o(\varepsilon^2)=\frac{\gamma}{\beta}\left(1+\frac{1}{(\delta_1-\delta_2)}(\delta_2 e^{\delta_1\tau}-\delta_1 e^{\delta_2\tau})\right)$$

$$+\frac{e_0}{s_0}\left(\frac{1}{\delta_1-\delta_2}(F(\tau)e^{\delta_1\tau}-G(\tau)e^{\delta_2\tau})-(Ae^{2\delta_1\tau}+Be^{2\delta_2\tau}-Ce^{(\delta_1+\delta_2)\tau})\right)+o(\varepsilon^2),$$

$$(41)$$

where

$$A=\frac{\gamma^2\delta_2^2(1+\delta_1)}{\beta^2(\delta_1-\delta_2)^2(2\delta_1-\delta_2)},$$

$$B=\frac{\gamma^2\delta_1^2(1+\delta_2)}{\beta^2(\delta_1-\delta_2)^2(2\delta_2-\delta_1)},$$

$$C=\frac{\gamma^2\delta_1^2((2\delta_2+1)\delta_1+\delta_2)}{\beta^2(\delta_1-\delta_2)^2},$$

$$F(\tau)=\frac{1}{\delta_1-\delta_2}\left((n\delta_2-m)+\frac{\gamma^2((\delta_1-\delta_2)\tau+1)}{\beta(\delta_1-\delta_2)^2}\right),$$

and

$$G(\tau)=\frac{1}{\delta_1-\delta_2}\left((n\delta_1-m)+\frac{\gamma^2((\delta_1-\delta_2)\tau-1)}{\beta(\delta_1-\delta_2)^2}\right).$$

In the same way, the derivative of P also can be expanded as

$$\frac{dP}{d\tau}=\frac{dP_0}{d\tau}+\varepsilon\frac{dP_1}{d\tau}+o(\varepsilon^2)$$

$$=\frac{\gamma}{(\delta_1-\delta_2)}(e^{\delta_1\tau}-e^{\delta_2 t})+\frac{e_0}{s_0}\frac{dP}{d\tau}.$$

$$(42)$$

When we compare Eqs. (23) and (25) with Eqs. (41) and (42), we can conclude that even though the latter is closer to real solution, in the application point of view, it is complicated and, the nonlinear least-square fit may not yield any new information about the system. But when we compare Eq. (8) with Eqs. (23) and (25), it is clear that the latter gives very good approximation closer to the real solution and also satisfies the condition given by Eq. (9).

SIMULATION OF SYSTEM (1)

Since the time evolution of [ES] has turnover behavior, in order to check the validity of solution (30), we can choose the point $(t_m, [ES]_{max})$ to compare with simulated data. Theoretical t_m can be calculated by equating the time derivative of [ES] to zero

TABLE I. Simulation results.

s_0 (μM)	e_0 (μM)	t_m(s) (Simulated)	t_m(s) (Predicted)	[ES]$_{max}$ (simulated) (μM)	Percentage error in predicted t_m
1	0.01	6.6750	6.6147	0.0094	0.9
1	0.10	4.6580	4.4040	0.0920	5.0
1	1.00	3.1210	1.9730	0.6634	36.0
0.1	1.00	2.8360	2.6360	0.0792	7.0

$$\frac{d[ES]}{dt} = \frac{dx}{dt} = \frac{1}{k_3}\frac{d^2 p_0}{dt^2}$$

$$= \frac{k_1 s_0 e_0}{\sqrt{\alpha^2 - 4\beta}}(\delta_2 e^{\delta_1 k_3 t_m} - \delta_1 e^{\delta_2 k_3 t_m}) = 0,$$

$$\tag{43}$$

$$t_m = \frac{1}{k_3\sqrt{\alpha^2 - 4\beta}}\ln\left(\frac{\delta_2}{\delta_1}\right), \tag{44}$$

or from Eq. (32),

$$t_m = \frac{2}{k_3\sqrt{\alpha^2 - 4\beta}}\tanh^{-1}\left(\frac{\sqrt{\alpha^2 - 4\beta}}{\alpha}\right). \tag{45}$$

A series of stochastic simulations of system (1) were done using CKS simulator (Version 1.01), IBM free software) with the following settings: The total number of molecules of 10 000 enabled an equilibrium detection mode, the selection frequency of 90%, an equilibrium test cycle length of 100 events, and the values of simulation parameters chosen were: $k_1 = 1$ μM^{-1} s^{-1}, $k_2 = 2 \times 10^{-2}$ s^{-1}, $k_3 = 8 \times 10^{-3}$ s^{-1} and $0.01 \leq \varepsilon \leq 10$. From the generated [ES] data, τ_m and [ES]$_{max}$ were measured and compared with the predicted values [Eqs. (44) and (45)]. Theoretical K_m and V_{max} were calculated using Eq. (8) and t_m was calculated using Eqs. (44) and (45).

RESULTS AND DISCUSSIONS

Simulation results are given to Table I along with predicted t_m values and simulated data are shown in Fig. 1. The results clearly indicate that the percentage error in the predicted t_m by zeroth-order gauge function is much less in the case of $\varepsilon < 0.1$ and $\varepsilon > 1$. But when $\varepsilon = 1$, the error in the prediction of t_m is maximum (36%). In this condition, the perturbation is completely "on." So, we have to use Eqs. (41) and (42) to compute the t_m values. But since most of the biological conditions fall in the range of $(0 < \varepsilon \ll 1)$, the error due to the zeroth-order gauge function is tolerable in biological systems. Though the error is high in the case of $\varepsilon = 1$, when we compare [ES]$_{max} = 0.6634$ μM from simulation with SSA value of 0.9242 μM given by Eq. (8), the error is 40%. At the same time error in [ES]$_{max}$ due to zeroth-order gauge function (in this case, [ES]$_{max} = 0.49$ μM) is only 24%. Thus, Eqs. (29) and (30) not only well predicted the t_m value but also [ES]$_{max}$ better than SSA. The effect of t_c in an LB plot that leads to nonlinearity near origin has been shown in Fig. 2. From this plot, we can conclude that at a suffi-
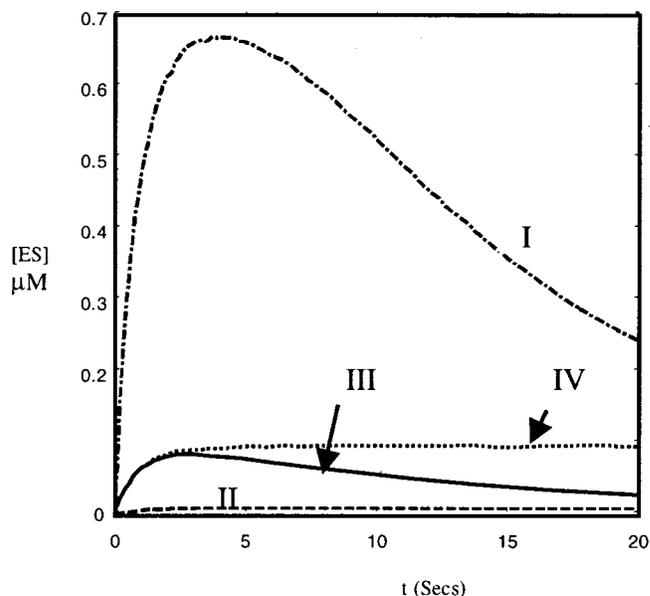

FIG. 1. The time evolution of [ES] from CKS simulation has been shown for different values of e_0 and s_0 and same $k_1 = 1$ μM^{-1} s^{-1}, $k_2 = 2 \times 10^{-3}$ s^{-1} and $k_3 = 8 \times 10^{-2}$ s^{-1}. (I)-$e_0 = 1$ μM, $s_0 = 1$ μM, (II)-$e_0 = 0.01$ μM, $s_0 = 1$ μM, (III)-$e_0 = 1$ μM, $s_0 = 0.1$ μM, and (IV)-$e_0 = 0.1$ μM, $s_0 = 1$ μM.

ciently large (in this case, $t_c > 12$ s) reaction time t_c, the nonlinearity will disappear.

CONCLUSIONS

A composite solution to Michaelis–Menten enzyme kinetic equation was obtained by ordinary perturbation methods up to the first-order level (using MAPLE7) and it was shown that the zeroth-order gauge function itself predicted t_m and [ES]$_{max}$ better than SSA by extensive stochastic simulations using the CKS simulator. The effect of transient dynamics on the linearity of the LB plot also was demonstrated.
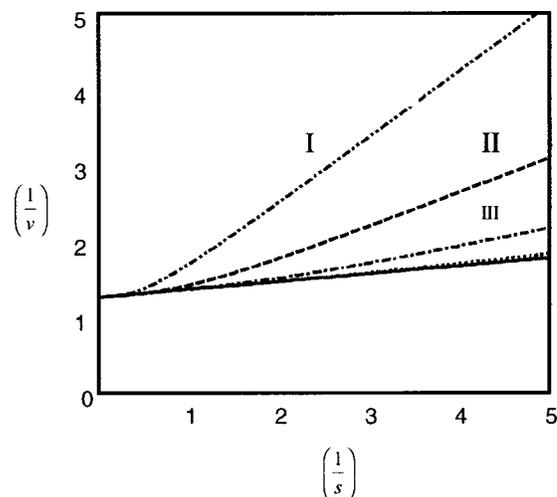

FIG. 2. The effect of reaction time t_c on double reciprocal plot has been shown. Here, $(1/V_{max}) = 12.5$ μM^{-1} s^{-1}, $(K_m/V_{max}) = 1.025$ s, $k_1 = 1$ μM^{-1} s^{-1}, $k_2 \times 10^{-3}$ s^{-1} and $k_3 = 8 \times 10^{-2}$ s^{-1} (I)-$t_c = 1.5$ s, (II)-$t_c = 3$ s, (III)-$t_c = 6$ s. Solid line indicates the ideal plot predicted by SSA.

ACKNOWLEDGMENTS

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