

Can One Measure All Rate Constants in the Simplest Enzyme Kinetics Model?

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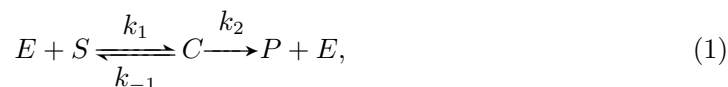
Abstract Enzymes play a vital role in life process and almost all biochemical reactions are mediated by enzymes. The rate constants of enzyme kinetics are the most important indexes for the reactions catalyzed by enzymes. In 1902, Adrian Brown proposed a simple single-substrate-single-product model which can be described by three rate constants k_1 , k_{-1} and k_2 . So far, biologists can measure the Michaelis constant K_M and the catalytic constant k_{cat} according to Michaelis-Menten equation. However, nobody has measured all the rate constants in this simple enzymatic model. In this article, we design three methods that could measure the rate constants k_1 and k_{-1} based on knowing k_{cat} and K_M . And our numerical experiments show that the three rate constants can be calculated rather precisely. Hence, we believe that biologists could design experiments to test our methods.

Key words enzyme kinetics, Michaelis constant, catalytic constant, Michaelis-Menten equation

1 Introduction

Enzymes play important roles in life processes[1]. They are involved in almost all the chemical reactions concerning the metabolism. The rates of reactions and the change of rates of reactions under different conditions cover information on functions and structures of enzymes. Moreover the chemical characters of reactants are often indicated by rate constants. Thus, the major task of enzyme kinetics is to determine the enzymatic reaction rates, which is a very important problem as stated in the famous text book[2].

Adrian Brown first studied the rates of hydrolysis of sucrose by the yeast enzyme β -fructofuranosidase in 1902, which is the first case study in enzyme kinetics[3]. During the study, he proposed the basic model for enzyme kinetics. In his model, there is only one substrate S , that is catalyzed by an enzyme E into a single product P . This reaction consists of two successive elementary reactions. In the first elementary reaction, the substrate and the enzyme form a complex called enzyme-substrate complex denoted by C , and the complex breaks down into product and enzyme in the second one. The following chemical reaction equation indicates this process:



where k_1 and k_{-1} , denote respectively the forward and reverse rate constants of the formation of the enzyme-substrate complex, and k_2 denote the rate constant of the decomposition of the complex into product and enzyme.

The reaction process can then be described by the following system of differential equations[4]:

$$\frac{dS(t)}{dt} = -k_1S(t)E(t) + k_{-1}C(t) \quad (2)$$

$$\frac{dE(t)}{dt} = -k_1S(t)E(t) + (k_{-1} + k_2)C(t) \quad (3)$$

$$\frac{dC(t)}{dt} = k_1S(t)E(t) - (k_{-1} + k_2)C(t) \quad (4)$$

$$\frac{dP(t)}{dt} = k_2C(t) \quad (5)$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0), \quad (6)$$

where $E(t)$, $S(t)$, $C(t)$ and $P(t)$ denote the concentrations of enzymes, substrates, enzyme-substrate complexes and products at time t during the process, respectively. Obviously, we have the following equalities:

$$E(t) + C(t) = E_0 \quad (7)$$

$$S(t) + C(t) + P(t) = S_0. \quad (8)$$

Owing to these equalities, the (2)-(5) is equivalent to one of the systems (31), (40) and (45).

As these systems of differential equations can not be explicitly integrated, many enzymologists added more conditions or assumptions on these systems to simplify this problem. Leonor Michaelis and Maud Menten added the condition that $k_{-1} \gg k_2$ in 1913[5]. However, this condition is usually unrealistic and has little usage. G.E. Briggs and John B.S. Haldane in 1925 proposed the famous assumption in enzyme kinetics, i.e. the Quasi-Steady-State Assumption(QSSA[6]) under a more general condition $S_0 \gg E_0$ [7]. There are lots of papers discussing the qualification of QSSA and solutions of these systems when QSSA does not hold[4,8,9,10,11,12,13,14,15], and some paper extending this model to discuss enzyme competition or inhibition[16,17,18].

By QSSA, the equations (2)-(5) can be approximately solved explicitly, and some relations between rate constants can be gotten. Especially, Briggs and Haldane got the famous Michaelis-Menten equation:

$$v_0 = \frac{V_{max}S}{K_M + S}, \quad (9)$$

where K_M is the Michaelis constant defined as $K_M = \frac{k_{-1}+k_2}{k_1}$, v_0 is the initial velocity of the reaction and $V_{max} = k_2E_0$ is called as the maximal velocity of the reaction in many papers. More precisely, V_{max} is the least upper bound of the velocity of the reaction while E_0 is given and S_0 varies from 0 to infinity. This velocity can not be reached but this velocity can be well approximated as S_0 is large enough. In fact, the Michaelis-Menten equation holds not only at assemble level of enzyme molecules, but also at single-molecule level by the statistical analysis of the stochastic behave of single-molecule enzyme catalysis[1]. In 1934,

Hans Lineweaver and Dean Burk found that the reciprocal form of the Michaelis-Menten equation

$$\frac{1}{v_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{S}, \quad (10)$$

can be used to calculate K_M and V_{max} [19]. Actually, experiments are performed to get different pairs of $\frac{1}{S}$ and $\frac{1}{v_0}$, and then, K_M and V_{max} follow. Since E_0 is given in these experiments, k_2 follows by the equality $V_{max} = k_2 E_0$.

There are many books discussing the estimations of k_2 and K_M based on the reciprocal form of Michaelis-Menten equation[2,16,20]. In addition to this method, some more effective methods are also considered[21,22,23,24]. Nowadays, kinetic data are commonly treated by computer using complicated statistical methods[2]. Any way, we could assume that k_2 and K_M have already been able to measure.

However, there is no paper discussing how to measure the exact values of k_1 and k_{-1} . By knowing K_M , we already know a relation between k_1 and k_{-1} . In order to measure all the three rate constants, we must find another relation between k_1 and k_{-1} . In this paper, we provide three methods to find such relations. Then, k_1 and k_{-1} can be completely calculated. Numerical experiments show that our methods are effective. Hence, interested biologists can use any of these methods to design experiments to measure all the rate constants.

Our methods are derived by rigorous mathematics. Mathematics has been applied to Biology in many directions, such as the application of game theory to evolution by Maynard Smith and chaos to ecology by Robert May[25,26,27]. In this paper, we use analysis of precise structure around a singularity point of a dynamical system. The qualitative theory of dynamical system has more than 100 years' history[28], and is applied to many fields of sciences including biology[29,30,31], but it seems that this paper is the first one to analyze precisely a singularity point of the dynamical systems coming from enzyme kinetics. Perhaps, the most surprising thing is that the result we got here can be checked by biochemical experiments.

This paper is organized as follows. In Section 2, three methods for measuring these constants are given. Section 3 gives some numerical experiments to test our methods. Section 4 gives some discussions about our methods and some suggestions to biologists for designing experiments. Section 5 is the conclusion section. The mathematical foundation of these methods are given in Appendix.

2 Methods to measure all the rate constants

In the single-substrate-single-product enzyme reaction (1), k_1 , k_2 and k_{-1} are three rate constants. There are many methods for determining the parameters of the Michaelis-Menten equation (i.e. V_{max} and K_M). By knowing V_{max} and K_M , we already know k_2 and a relation between k_1 and k_{-1} . So, what we need to evaluate k_1 and k_{-1} is another relation between k_1 and k_{-1} .

In the following, we give three different relations between k_1 and k_{-1} . Using any one of them, k_1 and k_{-1} can be gotten based on knowing K_M and k_2 .

2.1 The first method

To use the first method, we require that one can measure the concentrations of the substrate and enzyme at the moment near the end of the reaction, where the reaction is

described by equations

$$\frac{dE}{dt} = -k_1SE + (k_{-1} + k_2)(E_0 - E), \quad (11)$$

$$\frac{dS}{dt} = -k_1SE + k_{-1}(E_0 - E), \quad (12)$$

with the initial condition $(S(0), E(0)) = (S_0, E_0)$. We denote their concentrations by \hat{S} and \hat{E} , respectively.

Now, a new relation between k_1 and k_{-1} is given by

$$T_1 = -\frac{k_1E_0 - (k_{-1} + k_2) + \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}}{2k_{-1}}, \quad (13)$$

where $T_1 = \lim_{t \rightarrow +\infty} \frac{E(t) - E_0}{S(t)}$. (The detailed deduction of this relation is in Appendix 6.1.)

So, T_1 can be approximated by $\hat{T}_1 = \frac{\hat{E} - E_0}{\hat{S}}$, if \hat{S} and \hat{E} were measured at the moment \hat{t} near the end of the reaction (see Figure 1 and 2). Moreover, the nearer (\hat{S}, \hat{E}) approaches the end point $(0, E_0)$ of the reaction, the better $\hat{T}_1 = \frac{\hat{E} - E_0}{\hat{S}}$ approximates T_1 .

Then, by (13) and the following two equations

$$V_{max} = k_2E_0, \quad (14)$$

$$K_M = \frac{k_{-1} + k_2}{k_1}, \quad (15)$$

k_1 , k_2 and k_{-1} are solved as,

$$k_2 = \frac{V_{max}}{E_0}, \quad (16)$$

$$k_1 = \frac{(\frac{V_{max}}{E_0})T_1^2}{K_M(-T_1 + T_1^2) - E_0(-T_1 + 1)}, \quad (17)$$

$$k_{-1} = K_M \left(\frac{\frac{V_{max}}{E_0}T_1^2}{K_M(-T_1 + T_1^2) - E_0(-T_1 + 1)} \right) - \frac{V_{max}}{E_0}. \quad (18)$$

Since V_{max} and K_M are assumed known, k_1 , k_2 and k_{-1} are obtained from equations (16)-(18), if T_1 in them is replaced by $\hat{T}_1 = \frac{\hat{E} - E_0}{\hat{S}}$.

2.2 The second method

Here we give the second method, which requires measuring the concentrations of the substrate and product at the moment near the end of the reaction, where the reaction is described by equations

$$\frac{dS}{dt} = -k_1E_0S + k_1S_0S - k_1S^2 - k_1PS + k_{-1}S_0 - k_{-1}P - k_{-1}S, \quad (19)$$

$$\frac{dP}{dt} = -k_2S - k_2(P - S_0), \quad (20)$$

with the initial condition $(S(0), P(0)) = (S_0, 0)$. We denote their concentrations by \hat{S} and \hat{P} , respectively.

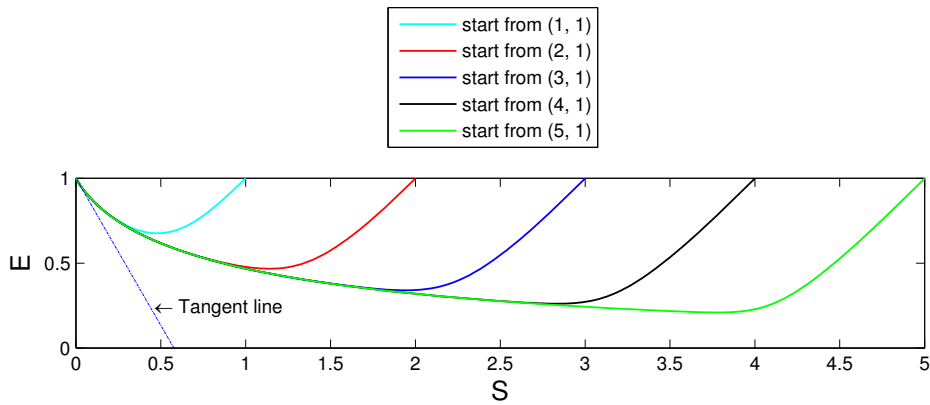


Figure 1: This graph shows that the trajectories of the concentrations of enzyme and substrate from different initial conditions have a common tangent line at the end point $(0, 1)$. In other words, the ratio of $\frac{E-E_0}{S}$ on each trajectory approximates the slope of the tangent line when S is sufficiently small.

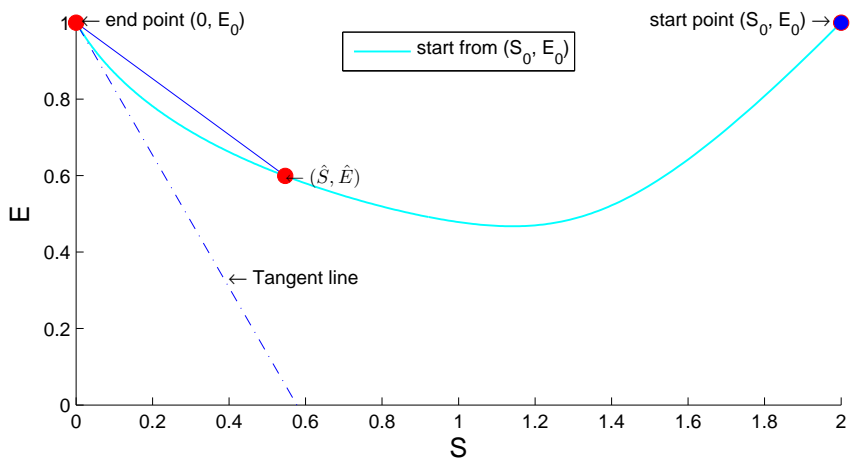


Figure 2: This graph indicates that the slope of the secant line between two red points is $\frac{\hat{E}-E_0}{\hat{S}}$, which approximates T_1 , the slope of the tangent line, when \hat{S} is sufficiently small.

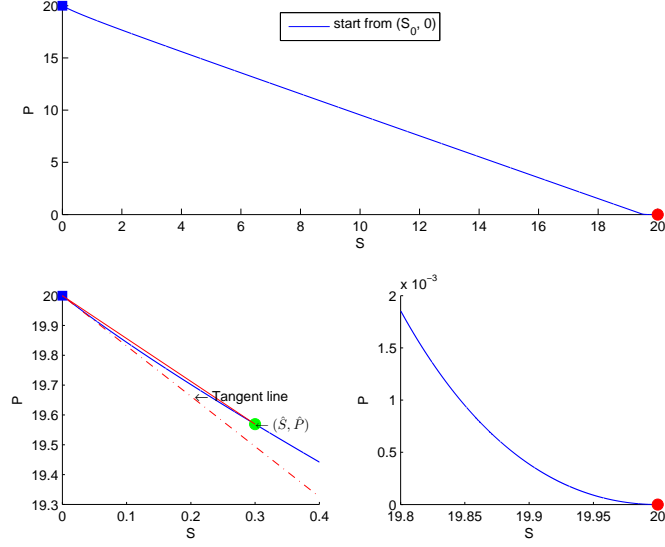


Figure 3: In this graph the red circle indicates the start point of the trajectory, the blue square is the end point and the green circle is the point (\hat{S}, \hat{P}) . The lower left panel of this graph indicates that the slope of the secant line between the green circle and the blue square is $\frac{\hat{P}-S_0}{\hat{S}}$, which approximates T_2 , the slope of the tangent line, when \hat{S} is sufficiently small. The lower right panel shows the trajectory near the start point.

Similarly, we give another new relation between k_1 and k_{-1} as follows,

$$T_2 = -\frac{2k_{-1}}{k_1 E_0 + k_{-1} - k_2 + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}, \quad (21)$$

where $T_2 = \lim_{t \rightarrow +\infty} \frac{S(t)}{P(t) - S_0}$.

By the equations (14), (15) and (21), the rate constants k_1 , k_2 and k_{-1} are solved as,

$$k_2 = \frac{V_{max}}{E_0}, \quad (22)$$

$$k_1 = \frac{(\frac{V_{max}}{E_0})(T_2 + 1)^2}{E_0 T_2 + K_M + K_M T_2}, \quad (23)$$

$$k_{-1} = K_M \left(\frac{(\frac{V_{max}}{E_0})(T_2 + 1)^2}{E_0 T_2 + K_M + K_M T_2} \right) - \frac{V_{max}}{E_0}. \quad (24)$$

In this method, T_2 is approximated by $\hat{T}_2 = \frac{\hat{S}}{\hat{P} - S_0}$, if \hat{S} and \hat{P} were measured at the moment \hat{t} near the end of the reaction. Similarly to the assertion in the first method, the nearer (\hat{S}, \hat{P}) approaches the end point $(0, S_0)$ of the reaction, the better the approximation is (see Figure 3).

2.3 The third method

In the third method, we require to measure the concentrations of the enzyme and product at the moment near the end of the reaction, where the reaction is described by

$$\frac{dP}{dt} = k_2(E_0 - E), \quad (25)$$

$$\frac{dE}{dt} = -k_1(S_0 - P - E_0 + E)E + (k_{-1} + k_2)(E_0 - E), \quad (26)$$

with the initial condition $(P(0), E(0)) = (0, E_0)$. We denote their concentrations by \hat{E} and \hat{P} , respectively.

Once more, we give another relation between k_1 and k_{-1} by

$$T_3 = \frac{2k_1E_0}{(k_1E_0 + k_{-1} + k_2) + \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}}, \quad (27)$$

where $T_3 = \lim_{t \rightarrow +\infty} \frac{E(t) - E_0}{P(t) - S_0}$.

Then, by (14), (15) and (27), the rate constants are solved as follows,

$$k_2 = \frac{V_{max}}{E_0}, \quad (28)$$

$$k_1 = \frac{(\frac{V_{max}}{E_0})T_3^2}{-E_0 + E_0T_3 + K_M T_3}, \quad (29)$$

$$k_{-1} = K_M \left(\frac{(\frac{V_{max}}{E_0})T_3^2}{-E_0 + E_0T_3 + K_M T_3} \right) - \frac{V_{max}}{E_0}. \quad (30)$$

In this method, T_3 is approximated by $\hat{T}_3 = \frac{\hat{E} - E_0}{\hat{P} - S_0}$, if \hat{E} and \hat{P} were measured at the moment \hat{t} near the end of the reaction. The nearer (\hat{E}, \hat{P}) approaches the end point (E_0, S_0) of the reaction, the better the approximation is.

3 Numerical simulations

In the above section, we mentioned that T_1 , T_2 and T_3 could be approximated by $\frac{\hat{E} - E_0}{\hat{S}}$, $\frac{\hat{S}}{\hat{P} - S_0}$ and $\frac{\hat{E} - E_0}{\hat{P} - S_0}$, respectively. And then, more importantly, the rate constants k_1 , k_2 and k_{-1} could be calculated.

So, in this section, we simulate the reaction processes by computers to show how to evaluate the three rate constants and how well our methods work. In the following simulations, we use four order Runge-Kutta method to calculate the reaction processes, and the step-length is set as 0.00002.

3.1 Numerical simulation of the first method

As stated in the first method, T_1 can be approximated by $\frac{\hat{E} - E_0}{\hat{S}}$ if \hat{S} is small enough. Here, we do some numerical experiments at first to show that T_1 can be well estimated if proper \hat{S} and its corresponding \hat{E} are measured.

Consider that the process of the reaction (1) can be described by equations (11) and (12) with the initial condition $(S(0), E(0)) = (S_0, E_0)$. After setting the rate constants

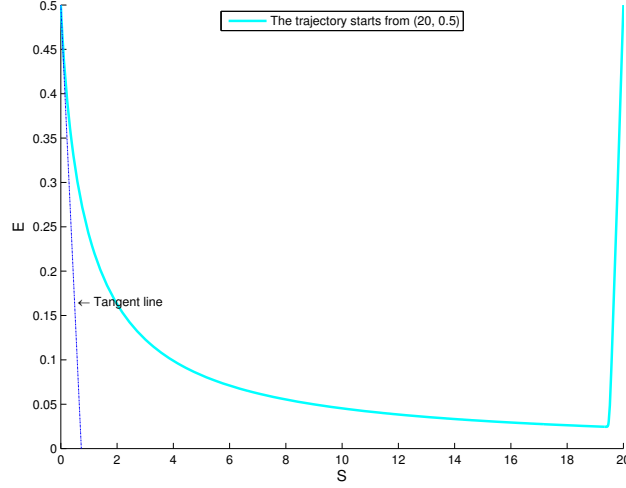


Figure 4: This graph shows the trajectory of the reaction (1) under setting the rate constants $k_1 = 0.3$, $k_2 = 0.2$ and $k_{-1} = 0.1$ and the initial condition $(S(0), E(0)) = (20, 0.5)$.

$k_1 = 0.3$, $k_2 = 0.2$ and $k_{-1} = 0.1$ and the initial condition $(S(0), E(0)) = (20, 0.5)$, the time evolutions of $E(t)$ and $S(t)$ can be calculated by numerical integration. The trajectory of $(S(t), E(t))$ is plotted in Figure 4.

In the above reaction,

$$T_1 = -\frac{k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2k_{-1}} = -0.686140661635.$$

Several pairs of (\hat{S}, \hat{E}) are chosen, and the corresponding values of \hat{T}_1 are calculated. The results are listed in Table 1. From the results, we see as expected that T_1 is well approximated by \hat{T}_1 as (\hat{S}, \hat{E}) approaches the end point $(0, E_0)$.

Since we have had the approximation of T_1 , that is \hat{T}_1 , the three rate constants k_1 , k_2 and k_{-1} can be solved by equations (16)-(18). To exclude the affections of the measuring deviations of K_M and V_{max} , we assume here that the exact values of K_M and V_{max} can be got by experiments. Now, any deviations between the estimations of k_1 , k_{-1} and k_2 and their exact values are caused by our methods. So we can test the effectiveness of our methods.

In this numerical experiment, we have $K_M = \frac{k_{-1} + k_2}{k_1} = 1$ and $V_{max} = k_2 E_0 = 0.1$. So the estimation of k_2 is obvious, that is $\hat{k}_2 = \frac{V_{max}}{E_0} = 0.2$. For different values of \hat{T}_1 , we have different \hat{k}_1 and \hat{k}_{-1} . Results show that our method provides better approximate values of \hat{k}_1 and \hat{k}_{-1} when \hat{T}_1 is more approximate to T_1 .

To give a more detail and straightforward description of our experiment results, we plot them in a graph (see Figure 5). From this graph, we can obviously see that T_1 , k_1 and k_{-1} can be well calculated as long as sufficiently small \hat{S} and its corresponding \hat{E} were measured exactly.

\hat{S}	\hat{E}	\hat{T}_1	\hat{k}_1	\hat{k}_2	\hat{k}_{-1}
0.04000	0.474866711865	-0.628332203365	0.377859173663	0.2	0.177859173663
0.03600	0.477197252368	-0.633409656440	0.368228137372	0.2	0.168228137372
0.03200	0.479564198907	-0.638618784145	0.359098307804	0.2	0.159098307804
0.02800	0.481968922796	-0.643967043011	0.350429845607	0.2	0.150429845607
0.02400	0.484412895900	-0.649462670828	0.342186722415	0.2	0.142186722415
0.02000	0.486897703412	-0.655114829414	0.334336203817	0.2	0.134336203817
0.01600	0.489425059325	-0.660933792165	0.326848385257	0.2	0.126848385257
0.01200	0.491996825596	-0.666931200318	0.319695758263	0.2	0.119695758263
0.00800	0.494615036530	-0.673120433742	0.312852773850	0.2	0.112852773850
0.00400	0.497281931202	-0.679517199447	0.306295335480	0.2	0.106295335480
0.00200	0.498634401594	-0.682799202842	0.303116383956	0.2	0.103116383956
0.00100	0.499315537699	-0.684462301039	0.301550563247	0.2	0.101550563247
0.00050	0.499657350227	-0.685299545383	0.300773400314	0.2	0.100773400314
0.00010	0.499931402802	-0.685971978298	0.300154516829	0.2	0.100154516829
0.00002	0.499986278786	-0.686060692395	0.300073216676	0.2	0.100073216676

Table 1: The first two columns of this table list the concentrations of \hat{S} and \hat{E} , respectively. The third column lists the estimated values of T_1 , which are denoted by \hat{T}_1 . And the last three columns give the corresponding estimations of k_1 , k_2 and k_{-1} .

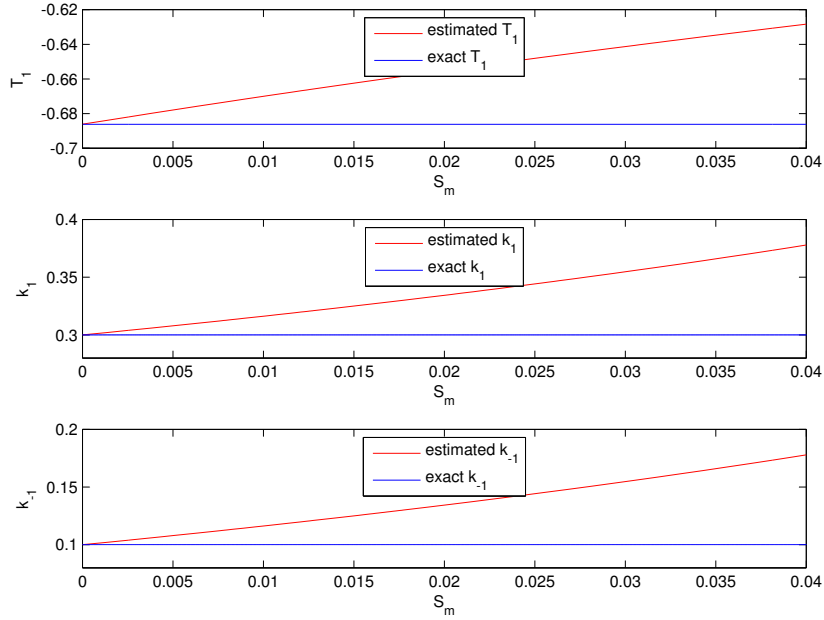


Figure 5: There are three panels in this graph. From top to bottom, they indicate the estimations of T_1 , k_1 and k_{-1} with respect to different values of \hat{S} from 0.04 to 0, respectively.

\hat{S}	\hat{P}	\hat{T}_2	\hat{k}_1	\hat{k}_2	\hat{k}_{-1}
0.04000	19.934866711865	-0.614125298224	0.377859173662	0.2	0.177859173662
0.03600	19.941197252368	-0.612216290051	0.368228137371	0.2	0.168228137371
0.03200	19.947564198907	-0.610270069937	0.359098307804	0.2	0.159098307804
0.02800	19.953968922796	-0.608284700263	0.350429845605	0.2	0.150429845605
0.02400	19.960412895900	-0.606258036441	0.342186722414	0.2	0.142186722414
0.02000	19.966897703412	-0.604187686696	0.334336203816	0.2	0.134336203816
0.01600	19.973425059325	-0.602070958347	0.326848385255	0.2	0.126848385255
0.01200	19.979996825596	-0.599904782998	0.319695758262	0.2	0.119695758262
0.00800	19.986615036530	-0.597685605790	0.312852773850	0.2	0.112852773850
0.00400	19.993281931202	-0.595409204699	0.306295335482	0.2	0.106295335482
0.00200	19.996634401594	-0.594247963934	0.303116383952	0.2	0.103116383952
0.00100	19.998315537699	-0.593661252839	0.301550563240	0.2	0.101550563240
0.00050	19.999157350227	-0.593366326321	0.300773400305	0.2	0.100773400305
0.00010	19.999831402802	-0.593129668146	0.300154516817	0.2	0.100154516817
0.00002	19.999966278786	-0.593098459956	0.300073216757	0.2	0.100073216757

Table 2: The first two columns of this table list the concentrations of \hat{S} and \hat{P} , respectively. The third column lists the estimated values of T_2 , which are denoted by \hat{T}_2 . And the last three columns give the corresponding estimations of \hat{k}_1 , \hat{k}_2 and \hat{k}_{-1} .

3.2 Numerical simulation of the second method

In this section, we use the time evolutions of the substrate $S(t)$ and product $P(t)$ to characterize the reaction process, which is different from that described by equations (11) and (12). Here, the trajectory is described by equations (19) and (20) with the initial condition $(S(0), P(0)) = (S_0, 0)$.

Similarly, we set the rate constants as $k_1 = 0.3$, $k_2 = 0.1$ and $k_{-1} = 0.1$, and the initial concentrations of substrate and enzyme as $S_0 = 20$ and $E_0 = 0.5$, respectively. Therefore, we have the trajectory by numerical integration of equations (19) and (20) (see Figure 3). In this reaction,

$$T_2 = -\frac{2k_{-1}}{k_1E_0 + k_{-1} - k_2 + \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}} = -0.593070330817.$$

We choose several pairs of (\hat{S}, \hat{P}) from the trajectory, and by which T_2 can be approximated by $\hat{T}_2 = \frac{\hat{S}}{\hat{P} - S_0}$, and thereby the approximations of the three rate constants k_1 , k_2 and k_{-1} can be calculated by the second method. The results are given by Table 2 and Figure 6.

These results also show that T_2 can be estimated accurately as long as \hat{S} is chosen small enough, and then k_1 , k_2 and k_{-1} can be well estimated.

By carefully examining the pairs of \hat{S} and \hat{E} in table (1) and the pairs of \hat{S} and \hat{P} in table (2), we find that the corresponding pairs with the same row number in these two tables are measured at the same time during the reaction process. Indeed, it is not a coincident. Because knowing any one pair among (\hat{S}, \hat{E}) , (\hat{S}, \hat{P}) and (\hat{P}, \hat{E}) yields the other two by the equations (7) and (8).

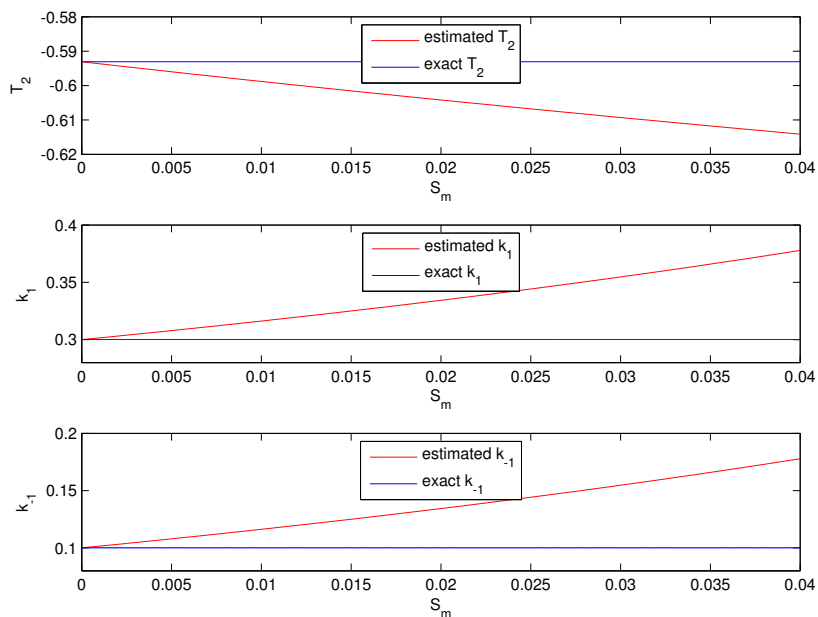


Figure 6: There are three panels in this graph. From top to bottom, they indicate the estimations of T_2 , k_1 and k_{-1} with respect to different values of \hat{S} from 0.04 to 0, respectively.

By comparing the results of the above two simulations, we find that if their corresponding measurements are done at the same time in a reaction, then the approximations of the rate constants are nearly same by the first two methods. And it is not hard to see that the results of the third method are the same, too. So, here we do not repeat such kind of simulations.

4 Discussion

So far, we have introduced three methods to evaluate the rate constants, and the results of our numerical experiments show the effectiveness of the methods. We claim that if biochemists can measure any pair among (\hat{S}, \hat{E}) , (\hat{S}, \hat{P}) and (\hat{P}, \hat{E}) in a single-substrate-single-product enzyme catalyzed reaction, the three rate constants can be calculated rather precisely. And theoretically the nearer to the end of the reaction the measurements are done, the more accurate the results are. This is also supported by the simulations in the last section. As listed in the Tables 1 and 2, when \hat{S} approaches 0, the approximations \hat{k}_1 , \hat{k}_2 and \hat{k}_{-1} approach their exact values.

However, we do not suggest to do measurements too close to the end of the reaction in experiments. Because if it is done too close to the end, any small measurement errors will lead to large errors in T_1 , T_2 or T_3 , and hence to large errors in k_1 and k_{-1} . Such kind of cases occur as well as in numerical simulations, if the precision of the simulation is low. Table 3 gives an example to show such cases.

For the numerical experiment of Table 3, the reaction processes of (11) and (12) are

\hat{S}	\hat{E}	\hat{T}_1	\hat{k}_1	\hat{k}_{-1}
0.220176690275	0.394933298085	-0.477192666415	-1.351779510969	-1.551779510969
0.099028990794	0.444020148082	-0.565287512969	0.625380786202	0.425380786202
0.004729967422	0.496791509356	-0.678332503834	0.307472825260	0.107472825260
0.000055635638	0.499961831471	-0.686044595056	0.300087962721	0.100087962721
0.000013762429	0.499990557363	-0.686117029880	0.300021629453	0.100021629453
0.000003223587	0.499997788187	-0.686134150984	0.300005958435	0.100005958435
0.000000392361	0.499999730637	-0.686518791813	0.299654687972	0.099654687972
0.000000089471	0.499999935983	-0.715513695675	0.276947107607	0.076947107607
0.000000028777	0.499999987301	-0.441281534834	-0.460190859183	-0.660190859183

Table 3: The first two columns of this table list the concentrations of \hat{S} and \hat{E} , respectively. The third column lists the estimated values of T_1 , which are denoted by \hat{T}_1 . And the last two columns give the corresponding estimations of \hat{k}_1 and \hat{k}_{-1} . In each cases, $\hat{k}_2 = 0.2$, so we do not list them in this table explicitly.

simulated by the Runge-Kutta-Fehlberg method in Matlab by setting $k_1 = 0.3$, $k_2 = 0.1$, $k_{-1} = 0.1$, $S_0 = 20$ and $E_0 = 0.5$.

In this simulation we see that, when some properly small \hat{S} and its correspondence \hat{E} were chosen, T_1 and then k_1 , k_2 and k_{-1} are calculated with small deviations from their exact values (see the rows 3 to 7 in the Table 3); while \hat{S} was too small, for the unavoidable errors of numerical integration, the errors of \hat{S} will lead to large errors in T_1 , k_1 and k_{-1} (see the last two rows in the Table 3). The details are shown in Table 3. Here we do not discuss the accuracy about k_2 , because in this article we assume K_M and V_{max} have been given exactly. Hence, k_2 is obtained exactly by $\frac{V_{max}}{E_0}$.

In the view point of theoretical analysis, the measurement should be done near the end of the reactions for the accuracy. But considering the unavoidable measurement errors, it should not be done too close to the end. So, some tradeoff should be considered in biochemical experiments.

Actually, to evaluate K_M and V_{max} accurately is still a challenge, which attracts many talent scientists to study. The good performance of our methods depends partially on evaluating K_M and V_{max} accurately. So, further study should focus on how to evaluate the rate constants k_1 , k_2 and k_{-1} accurately, without the assumption that K_M and V_{max} can be calculated exactly.

5 Conclusion

Enzyme kinetics, as an important branch of enzymology, is to study the rates of chemical reactions catalyzed by enzymes. So, how to measure the rate constants in enzyme catalyzed reaction naturally becomes a fundamental problem in enzyme kinetics. However, even for the simplest reaction schemed as (1), to measure all the three rate constants has bothered enzymologists since it came into being.

Before our current work, one can only measure V_{max} and K_M . In this article, we proposed three methods to calculate all the three rate constants in (1). Our methods are based on carefully analyzing the structure of integral curves near the singularity point in dynamical system. And it seems that this is novel in enzyme kinetics.

To test our methods we did some numerical analysis, and the results showed that these methods were effective. The accuracy of these three methods are almost the same, so choosing which one to use depends on the preference and convenience of biologists.

By the methods we proposed in this article, all the three rate constants can be calculated. Knowing all the rate constants should be helpful for deeper insights to the function and structure of enzymes. Furthermore, it should be also helpful to reveal the path followed by the reactants and gives more information about the reaction mechanism.

6 Appendix

It will be seen in this appendix that all the systems of differential equations we encountered are autonomous systems on the plane which have the singularity of the type stable nodal point. Moreover, the solutions, i.e. the integral curves of these systems under the initial conditions which have biological meaning will approach the singularity when time goes to infinity. And those integral curves must enter the singularity in the direction of the eigenvector with the smaller absolute value of the eigenvalue [32, p.90-94].

6.1 Arguments for the method in Section 2.1

Consider the system

$$\begin{cases} \frac{dS}{dt} = -k_1ES + k_{-1}(E_0 - E), \\ \frac{dE}{dt} = -k_1ES + (k_{-1} + k_2)(E_0 - E). \end{cases} \quad (31)$$

We have studied this system in our former paper[33]. Some part of it is useful here. For convenience to the readers, we rewrite the first three lemmas in that paper. Denote

$$\begin{aligned} P(S, E) &= -k_1ES + k_{-1}(E_0 - E), \\ Q(S, E) &= -k_1ES + (k_{-1} + k_2)(E_0 - E). \end{aligned}$$

The singularity of this system is $(S, E) = (0, E_0)$. The linearization of this system around this singularity is

$$\begin{cases} \frac{dS}{dt} \approx -k_1E_0S - k_{-1}(E - E_0), \\ \frac{dE}{dt} \approx -k_1E_0S - (k_{-1} + k_2)(E - E_0). \end{cases} \quad (32)$$

And its characteristic equation is

$$\lambda^2 + (k_1E_0 + k_{-1} + k_2)\lambda + k_1k_2E_0 = 0, \quad (33)$$

where λ are the eigenvalues and can be solved as

$$\lambda = \frac{-(k_1E_0 + k_{-1} + k_2) \pm \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}}{2}. \quad (34)$$

As both eigenvalues are negative, this singularity is a stable nodal point. The eigenvectors are

$$V_{\mp} = \left(k_{-1}, \frac{-k_1E_0 + k_{-1} + k_2 \mp \sqrt{(k_1E_0 + k_{-1} + k_2)^2 - 4k_1k_2E_0}}{2} \right).$$

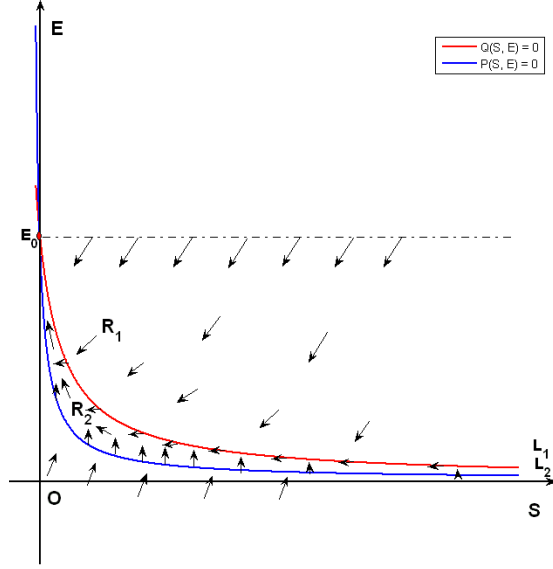


Figure 7: $Q(S, E) = 0$ and $P(S, E) = 0$ are two hyperbolas, which intersect each other at the point $(0, E_0)$. L_1 and L_2 are the two hyperbolas with $S \geq 0$, respectively. R_1 is the region above L_1 , and R_2 is between L_1 and L_2 . The arrows show the vector fields of dynamical system (31).

We will prove that the solution of (31) with initial condition $(S(0), E(0)) = (S_0, E_0)$ denoted by $(S(t), E(t))$ will approach the singularity in the direction of V_- . To prove this, we recall first some notations introduced in [33]. Let L_1 , L_2 , R_1 and R_2 be the point sets (see Figure 7)

$$\begin{aligned}
L_1 &= \{(S, E) : Q(S, E) = 0, S \geq 0\}, \\
L_2 &= \{(S, E) : P(S, E) = 0, S \geq 0\}, \\
R_1 &= \{(S, E) : E > \tilde{E}, (S, \tilde{E}) \in L_1\}, \\
R_2 &= \{(S, E) : \tilde{E} > E > \hat{E}, (S, \tilde{E}) \in L_1, (S, \hat{E}) \in L_2\}.
\end{aligned}$$

Notice that L_1 and L_2 are the hyperbolas $Q(S, E) = 0$ and $P(S, E) = 0$ with $S \geq 0$ respectively, and they intersect each other at the point $(0, E_0)$ (see Figure 7). From system (31), it is easy to deduce that

$$\begin{cases} \frac{dS}{dt} = P(S, E) < 0 \\ \frac{dE}{dt} = Q(S, E) < 0 \end{cases} \quad (35)$$

in the region R_1 ,

$$\begin{cases} \frac{dS}{dt} = P(S, E) < 0 \\ \frac{dE}{dt} = Q(S, E) > 0 \end{cases} \quad (36)$$

in the region R_2 ,

$$\begin{cases} \frac{dS}{dt} = P(S, E) < 0 \\ \frac{dE}{dt} = Q(S, E) = 0 \end{cases} \quad (37)$$

on the curve L_1 and

$$\begin{cases} \frac{dS}{dt} = P(S, E) = 0 \\ \frac{dE}{dt} = Q(S, E) > 0 \end{cases} \quad (38)$$

on the curve L_2 .

The corresponding vector fields can be deduced by the systems of equations (35)-(38) (see Figure 7).

Lemma 1: The solution $(S(t), E(t))$ of system (31) will arrive at the curve L_1 at some time $T_0 > 0$.

Proof: Firstly, we prove that $(S(t), E(t))$ will actually arrive at the hyperbola $Q(S, E) = 0$, where $\frac{dE}{dt} = 0$. Otherwise, there exists a positive number $\mu > 0$ such that $Q(S(t), E(t)) < -\mu$ for all $t > 0$. Therefore, $E(t)$ would decrease to zero at some time t_0 , which is less than $\frac{E_0}{\mu}$. However, $\frac{dE}{dt} = Q(S(t_0), 0) = (k_{-1} + k_2)E_0 > 0$ at time $t = t_0$, contradicting the fact that $\frac{dE}{dt} = Q(S(t), E(t)) < -\mu$.

Then let the arrival time be T_0 . If $S(T_0) \geq 0$, the proof is complete. If $S(T_0) < 0$, then $E(T_0) > E_0$ by the hyperbolic property of L_1 . Therefore, there must exist some $0 < \hat{t} < T_0$ such that $\frac{dE}{dt}E(\hat{t}) > 0$ for the initial value $E(0) = E_0$. This contradicts the fact that $\frac{dE}{dt}E(t) < 0$ for $0 \leq t < T_0$. Hence the solution $(S(t), E(t))$ of system (31) will arrive at L_1 at some time $T_0 > 0$. \square

Lemma 2: $E(t)$ and $S(t)$ decrease monotonously with respect to t from $t = 0$ until $(S(t), E(t))$ arrives at L_1 .

Proof: Since the solution $(S(t), E(t))$ of system (31) starts from (S_0, E_0) in R_1 at $t = 0$, $E(t)$ and $S(t)$ will decrease monotonously until $(S(t), E(t))$ arrives at L_1 by inequalities (35). \square

By inequalities (37), the solution $(S(t), E(t))$ crosses L_1 horizontally once it arrives at L_1 at the time T_0 . Then, $(S(t), E(t))$ will stay in the region R_2 permanently and approach the stable nodal point $(0, E_0)$ from the inequalities (36)-(38). Thus, the following lemma has been proved.

Lemma 3: Both $E(t)$ and $S(t)$ will decrease until $(S(t), E(t))$ horizontally crosses L_1 and enters the region R_2 . After that, $(S(t), E(t))$ will stay in R_2 and not attach L_1 or L_2 forever. In the region R_2 , $S(t)$ decreases and $E(t)$ increases continuously. At last, $(S(t), E(t))$ will approach the point $(0, E_0)$.

Therefore, the solution will enter the singularity $(0, E_0)$ in the direction of an eigenvector. For $E(t) < E_0$ and $S(t) > 0$ when $t > 0$, it must be $\frac{E(t) - E_0}{S(t)} < 0$, which proves that $(S(t), E(t))$ enters $(0, E_0)$ in the direction of V_- . Hence,

$$\lim_{t \rightarrow +\infty} \frac{E(t) - E_0}{S(t)} = -\frac{k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2k_{-1}}. \quad (39)$$

6.2 Arguments for the method in Section 2.2

This time we consider the system consisting of S and P

$$\begin{cases} \frac{dS}{dt} = -k_1(S + P + E_0 - S_0)S + k_{-1}(S_0 - S - P), \\ \frac{dP}{dt} = k_2(S_0 - S - P). \end{cases} \quad (40)$$

The singularity of this system is $(0, S_0)$.

The linearization of this system around this singularity is

$$\begin{cases} \frac{dS}{dt} \approx -k_1 E_0 S - k_{-1} S - k_{-1}(P - S_0), \\ \frac{dP}{dt} = -k_2 S - k_2(P - S_0). \end{cases} \quad (41)$$

Thus, its characteristic equation is

$$\lambda^2 + (k_1 E_0 + k_{-1} + k_2)\lambda + k_1 k_2 E_0 = 0, \quad (42)$$

where λ are the eigenvalues and are solved as

$$\lambda = \frac{-(k_1 E_0 + k_{-1} + k_2) \pm \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2}. \quad (43)$$

Since both eigenvalues are negative, this singularity is a stable nodal point, and the eigenvectors are

$$W_{\mp} = \left(k_{-1}, \frac{k_2 - k_1 E_0 - k_{-1} \mp \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2} \right).$$

Let $(S(t), P(t))$ denote the solution of (40) with condition $(S(0), P(0)) = (S_0, 0)$. Note that (31) and (40) are equivalent. According to what we have proved in section 6.1, $\lim_{t \rightarrow +\infty} S(t) = 0$ and $\lim_{t \rightarrow +\infty} E(t) = E_0$. Therefore, by (7) and (8), it is easy to verify that $\lim_{t \rightarrow +\infty} P(t) = S_0$. The solution of system (40) with initial condition $(S(0), P(0)) = (S_0, 0)$ will approach the singularity $(0, S_0)$. Moreover, its approaching is in the direction of an eigenvector. As equations (7), (8) and Lemma 3 stated, it is easy to conclude that $\lim_{t \rightarrow +\infty} \frac{S(t)}{P(t) - S_0} \leq 0$, i.e. such approaching is in the direction of W_- . Thus,

$$\lim_{t \rightarrow +\infty} \frac{S(t)}{P(t) - S_0} = -\frac{2k_{-1}}{k_1 E_0 + k_{-1} - k_2 + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}. \quad (44)$$

6.3 Arguments for the method in Section 2.3

Now we consider the system consisting of P and E

$$\begin{cases} \frac{dP}{dt} = k_2(E_0 - E), \\ \frac{dE}{dt} = -k_1 E(S_0 - P - E_0 + E) + (k_{-1} + k_2)(E_0 - E), \end{cases} \quad (45)$$

whose singularity is (S_0, E_0) .

The linearization of (45) around this singularity is

$$\begin{cases} \frac{dP}{dt} = -k_2(E - E_0), \\ \frac{dE}{dt} \approx -(k_1 E_0 + k_{-1} + k_2)(E - E_0) + k_1 E_0(P - S_0). \end{cases} \quad (46)$$

Hence, its characteristic equation is

$$\lambda^2 + (k_1 E_0 + k_{-1} + k_2)\lambda + k_1 k_2 E_0 = 0, \quad (47)$$

where λ are the eigenvalues and are solved as

$$\lambda = \frac{-(k_1 E_0 + k_{-1} + k_2) \pm \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2}. \quad (48)$$

Since both eigenvalues are negative, this singularity is a stable nodal point, and the eigenvectors are

$$U_{\mp} = \left(k_2, \frac{k_1 E_0 + k_{-1} + k_2 \mp \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}{2} \right).$$

$(P(t), E(t))$ is the solution of (45) with condition $(P(0), E(0)) = (0, E_0)$ since (31), (40) and (45) are equivalent. According to what we have proved in sections 6.1 and 6.2, $\lim_{t \rightarrow +\infty} P(t) = S_0$ and $\lim_{t \rightarrow +\infty} E(t) = E_0$, i.e. the solution of system (45) with initial condition $(P(0), E(0)) = (0, E_0)$ will approach the singularity (S_0, E_0) . Moreover, the approaching is in the direction of an eigenvector. Similar to section 6.2, it is easy to conclude that $\lim_{t \rightarrow +\infty} \frac{E(t) - E_0}{P(t) - S_0} \geq 0$ and the approaching is in the direction of U_- . Hence,

$$\lim_{t \rightarrow +\infty} \frac{E(t) - E_0}{P(t) - S_0} = \frac{2k_1 E_0}{(k_1 E_0 + k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}}. \quad (49)$$

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