

An improved method to measure all rate constants in the simplest enzyme kinetics model

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Abstract Since Adrian Brown and Victor Henri's work, the simplest enzyme kinetics model, which contains only three rate constants k_1 , k_2 and k_{-1} in 1902, has been thoroughly explored in many directions. By using the Michaelis–Menten equation, K_M and k_2 can be measured quickly. All the three rate constants can be derived by temperature jump method or transient state kinetics, but both methods need more complicated techniques and equipments. In our previous paper (Li et al. in J Math Chem 46:290–301, 2009), we gave a method to measure all the rate constants which does not require any additional equipment other than those needed for measuring K_M and k_2 . Here, we propose a new one which needs no additional equipment either. This method is based on a study of inflection points of integral curves. Numerical results show that the new one is much better than the previous one in two aspects: near the end of the reaction, the new one gives more accurate estimation; during the quasi-steady state of the reaction, it also gives good estimations while the previous one can not. Hence, this method not only advances the estimation accuracy, but also has more choices for measuring.

Keywords Michaelis–Menten equation · Rate constants of enzyme kinetics · Basic enzyme kinetics model

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1 Introduction

Enzymes are biochemical catalysts [1]. Almost all chemical reactions in life processes are mediated by enzymes. So studying enzymes can help people understand life processes well [2]. Enzyme kinetics is the discipline that studies the velocity of chemical reactions catalyzed by enzymes, which plays a vital role in the study of enzymes. Since the reaction velocity can be characterized by the rate constants, the major task of enzyme kinetics is to measure the rate constants of reactions catalyzed by enzymes [1].

Adrian Brown took the first case study of enzyme kinetics in 1902 [3]. Victor Henri proposed two reaction mechanisms which contains only one substrate and one product forming a substrate-enzyme complex [4,5]. One of these models became the basic model of enzyme kinetics. It consists of two elementary steps. In the first step, the substrate S and the enzyme E form a substrate-enzyme complex C with rate k_1 . This step is reversible with the rate constant k_{-1} . The substrate-enzyme complex C decomposes into product P and enzyme E with rate constant k_2 in the second step which is assumed to be irreversible.



Thoroughly studying this model can help us deal with more complex ones.

Since enzyme kinetics is a branch of chemical kinetics [1], law of mass action is usually applicable for modeling the reaction process. Then, the reaction process can be described by a system of differential equations [6]:

$$dS/dt(t) = -k_1 S(t)E(t) + k_{-1}C(t) \quad (2)$$

$$dE/dt(t) = -k_1 S(t)E(t) + (k_{-1} + k_2)C(t) \quad (3)$$

$$dC/dt(t) = k_1 S(t)E(t) - (k_{-1} + k_2)C(t) \quad (4)$$

$$dP/dt(t) = k_2 C(t) \quad (5)$$

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.

This system of equations is nonlinear, and can not be integrated explicitly. Many enzymologists added more conditions or assumptions on these systems to simplify this problem. In 1913, Michaelis and Menten proposed the equilibrium assumption that under the condition $k_{-1} \gg k_2$, the first step of this reaction reaches equilibrium [7]. That is to say

$$E(t)S(t)/C(t) = k_{-1}/k_1 = K. \quad (7)$$

which leads to:

$$v = dP/dt = k_2C(t) = k_2E_0S(t)/(S(t) + K), \quad (8)$$

where v is the velocity of the production P .

However, the condition in this assumption is usually unrealistic and has little usage. After that, Briggs and Haldane proposed the famous Quasi-Steady-State Assumption(QSSA) in 1925 [8], which is proved to be always true more than 80 years later and named as Quasi-Steady-State Law(QSSL) [9]. The assumption said [1]:

“Under the physiologically common condition that substrate is in great excess over enzyme ($S_0 \gg E_0$), the enzyme-substrate complex C remains approximately constant until the substrate is nearly exhausted with an exception of the transient initial stage of the reaction.”

That is to say

$$dC(t)/dt = k_1S(t)E(t) - (k_{-1} + k_2)C(t). \quad (9)$$

Hence, the velocity v of the production P is

$$v = dP/dt = k_2C(t) = k_2E_0S(t)/(S(t) + K_M), \quad (10)$$

where $K_M = (k_{-1} + k_2)/k_1$. Briggs and Haldane then got the equation:

$$v_0 = V_{\max}S_0/(K_M + S_0), \quad (11)$$

where v_0 is the initial velocity of the reaction and $V_{\max} = k_2E_0$. S_0 and E_0 are the initial concentrations of substrate and enzyme, respectively.

Note that, the forms of Eqs. (10) and (8) are similar. In order to recognize the importance of the pioneer work of Michaelis and Menten, Eq. (11) is known as Michaelis–Menten equation. This is the basic equation in enzyme kinetics.

Using the reciprocal form of the Michaelis–Menten equation, Lineweaver and Burk provided a method to measure K_M and V_{\max} [10]. From then on, many biologists discussed the validity of Michaelis–Menten equation and the exactness of the reciprocal form estimation [11–20]. The Michaelis–Menten equation is considered to provide a good relationship among these rate constants. At the single-molecule level, the enzyme molecule moves according to thermal fluctuation and reacts stochastically with substrate molecules [21,22]. By the statistical analysis of the stochastic behavior, Michaelis–Menten equation also holds [2,23]. However, the reciprocal form estimation provides relative poor accuracy [24]. In spite of this, many textbooks introduce it as an important method for its simplicity [1,25–27]. It provides an acceptable estimation in the era without computers. Nowadays, kinetics data are often treated by complicated statistical methods with computers, such as nonlinear regression [1].

Some more effective methods are provided to measure K_M and V_{\max} [14–16]. All these methods do not require additional equipments beyond those required by the method provided by Lineweaver and Burk. They just measure the concentrations of

enzymes and substrates during the reaction process. So we confirm that K_M and V_{\max} can be measured exactly.

Nowadays, biologists can use temperature jump method or transient state kinetics to measure all the rate constants k_1 , k_2 and k_{-1} . But these methods do require additional complicated equipments. Recently, we provided a new approach to measure all the rate constants without using any additional equipment other than those needed for measuring K_M and V_{\max} [28]. It only provides one more relation among these constants, which actually is the tangent line of integral curves at the end of the reaction [28].

In this paper, we do deeper analysis of the phase plane to provide a new method based on another relation, which is yielded by inflection points of integral curves. Numerical experiments show that this new method is much better than the previous one in two aspects: near the end of the reaction, the new one gives more accurate estimation than the previous one; during the quasi-steady state of the reaction, it also gives good estimations while the previous one can not be applied at all.

The motivation to yield this new relation comes from mathematical observation, that is, we observe that a curve formed by inflection points of some integral curves is more close to the real reaction process than the tangent line used in [28]. This new relation is an absolutely new discovery which former scientists never pay any attention to, and its value has not been recognized completely yet. It deserves more attention in future works.

The application of differential equations and dynamical systems into biology can be found in many fundamental text books on mathematical biology such as [29,30].

This article is organized as follows. Section 2 provides the method and Sect. 3 gives the numerical experiments. The derivation of the key equation to our method is given in Sect. 4, then the conclusion comes in Sect. 5.

2 Method

In the basic model, there are three rate constants k_1 , k_2 and k_{-1} . Knowing K_M and V_{\max} , we already get two relations of the rate constants, that is

$$V_{\max} = k_2 E_0, \tag{12}$$

$$K_M = (k_{-1} + k_2)/k_1. \tag{13}$$

To get the exact values of k_1 , k_2 and k_{-1} , we only need one additional independent relation. Here is the relation:

$$E(E_0 - E)[k_1 S E - k_{-1}(E_0 - E)] + S E_0[k_1 S E - (k_{-1} + k_2)(E_0 - E)] \approx 0, \tag{14}$$

where E and S represent any pair of the concentrations after the initial transient period. This is a linear relation of k_1 , k_2 and k_{-1} and can be written as

$$(SE^2(E_0 - E) + S^2EE_0)k_1 - (E(E_0 - E)^2 + SE_0(E_0 - E))k_{-1} - SE_0(E_0 - E)k_2 \approx 0 \tag{15}$$

Equation (14) is the key equation to our method, so we will give detailed explanations about it in Sect. 4.

By Eqs. (12–14), k_1 , k_2 and k_{-1} are solved as

$$k_1 \approx E(E_0 - E)^2 V_{\max}/B \quad (16)$$

$$k_2 = V_{\max}/E_0 \quad (17)$$

$$k_{-1} \approx (V_{\max}(-SE^2E_0 + SE^3 - S^2EE_0 + SE_0K_M(E_0 - E)))/B \quad (18)$$

where $B = E_0(-SE^2E_0 + SE^3 - S^2EE_0 + E(E_0 - E)^2K_M + SE_0K_M(E_0 - E))$. Since V_{\max} and K_M are assumed to be known, estimates of k_1 , k_2 and k_{-1} are obtained from (16–18) if any one pair of S and E is measured at the same time. Thus, the only thing left is to design experiments to measure one pair of S and E . Numerical results show that the estimations are very accurate when measurements are done near the end or during the quasi-steady state of the reaction. The detailed results are shown in Sect. 3.

3 Numerical experiment

The whole process of the reaction ($S(t)$, $E(t)$) can be drawn on the $S - E$ plane. The equation

$$E(E_0 - E) [k_1SE - k_{-1}(E_0 - E)] + SE_0 [k_1SE - (k_{-1} + k_2)(E_0 - E)] = 0, \quad (19)$$

which relates to (14), defines a curve on the $S - E$ plane. It is an essential point of our new method that the curve (19) approximates the real reaction process well. In [28], we use the tangent line to approximate the real reaction when the reaction is near its end. Fig. 1 illustrates a case calculated by computers, which shows that the red curve, that is the approximation curve (19) proposed in this paper, is much better than the tangent line after the initial period. To show this more convincing, we do the same experiment as in [28] to compare these two methods.

We simulate the reaction processes by fourth order Runge–Kutta method with step-length 0.00002 on computers. In fact, the whole process can be described by the following equations [16]:

$$dS/dt = -k_1SE + k_{-1}(E_0 - E), \quad (20)$$

$$dE/dt = -k_1SE + (k_{-1} + k_2)(E_0 - E) \quad (21)$$

with the initial condition ($S(0)$, $E(0)$) = (S_0 , E_0). We set $k_1 = 0.3$, $k_2 = 0.2$, $k_{-1} = 0.1$ and (S_0 , E_0) = (20, 0.5), which are the same settings as in [28]. Then, ($S(t)$, $E(t)$) can be calculated for $t > 0$.

In this numerical experiment, we also assume that K_M and V_{\max} have already been measured exactly. That is to say, $K_M = (k_{-1} + k_2)/k_1 = 1$ and $V_{\max} = k_2E_0 = 0.1$. So $k_2 = V_{\max}/E_0 = 0.2$.

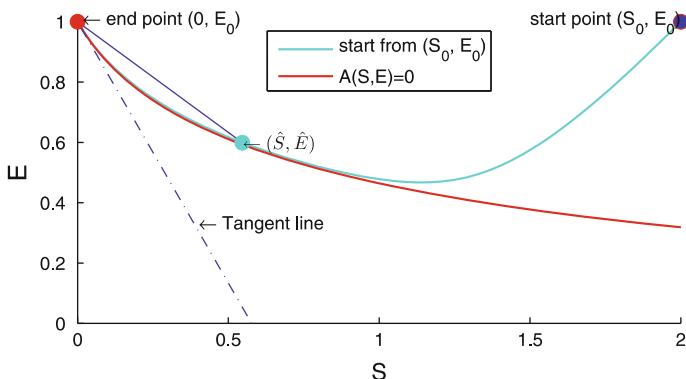


Fig. 1 Approximation of the real reaction process: the blue curve indicates the real reaction process. The dash line represents the tangent line, and the red curve is the approximate curve proposed in this paper (Color figure online)

Table 1 The first two columns of this table list the concentrations of S and E , respectively

S	E	\hat{k}_1	\tilde{k}_1	\hat{k}_{-1}	\tilde{k}_{-1}
0.0400	0.4749	0.3779	0.3060	0.1779	0.1060
0.0360	0.4772	0.3682	0.3055	0.1682	0.1055
0.0320	0.4796	0.3591	0.3050	0.1591	0.1050
0.0280	0.4820	0.3504	0.3045	0.1504	0.1045
0.0240	0.4844	0.3422	0.3039	0.1422	0.1039
0.0200	0.4869	0.3343	0.3034	0.1343	0.1034
0.0160	0.4894	0.3268	0.3028	0.1268	0.1028
0.0120	0.4920	0.3197	0.3021	0.1197	0.1021
0.0080	0.4946	0.3129	0.3015	0.1129	0.1015
0.0040	0.4973	0.3063	0.3008	0.1063	0.1008
0.0020	0.4986	0.3031	0.3004	0.1031	0.1004
0.0010	0.4993	0.3016	0.3002	0.1016	0.1002
0.0005	0.4997	0.3008	0.3001	0.1008	0.1001
0.0001	0.4999	0.3002	0.3000	0.1002	0.1000

\hat{k}_1 and \hat{k}_{-1} represent the estimated value calculated by former method. \tilde{k}_1 and \tilde{k}_{-1} represent the estimated value calculated by the new method, i. e. the righthand side of (16) and (18), respectively

As mentioned in the above section, to get the approximate values of k_1 and k_{-1} , we need to measure just one pair of S and E at the same time during the reaction process. In this experiment, we choose any pair of S and E near the end of the reaction, and substitute them in (16) and (18). Table 1 gives a few results.

In [28], the nearer the end of the reaction the measurements are done, the more accurate the results are. However, if the measurement is done too close to the end of the reaction, the unavoidable measurement error may lead to large errors in k_1 , k_2 and k_{-1} . Near the end of the reaction, we see from Table 1 that this new method does much better. For example, the result got by the new method for $S = 0.04$ is better than that

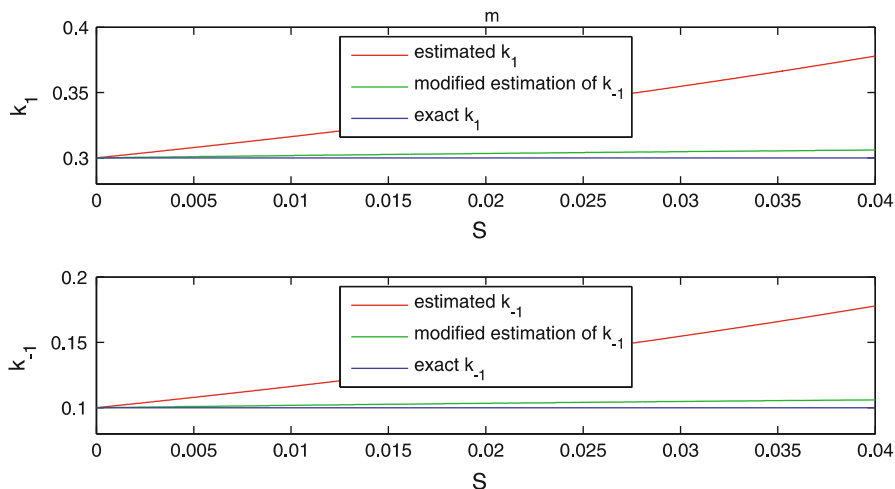


Fig. 2 A much better estimation: the *red line* shows the estimation in [28] and the *green one* shows the estimation in this article (Color figure online)

got by the previous one even for $S = 0.004$. That means, using the new method, we need not measure concentrations of reactants so close to the end of the reaction as the previous one.

To give a more illustrative description, we draw the results of these two methods in Fig. 2.

In [9], we have proved two quasi-steady state laws, which show that the reaction would attain a quasi-steady state after the initial transient period provided $S_0 \gg E_0$. This new method can also be applied during the quasi-steady state of the reaction. To give an example, we test it on the reaction mentioned above, that is the reaction with rate constants $k_1 = 0.3$, $k_2 = 0.2$, $k_{-1} = 0.1$ and initial condition $(S_0, E_0) = (20, 0.5)$. Some results are listed in Table 2, which show that when $S_0 \gg E_0$, during the quasi-steady state of the reaction, the new method gives very good estimations of the rate constants. The reason for this appearance is given in Sect. 4. Note that the first two estimations of the left column in Table 2 are absurd. This is because the new relation (19) differs with the real reaction largely during the initial transient period. Thus, this method should be applied after the initial transient period.

An intuitive description of rate constants estimations is shown in Fig. 3. This figure shows again that our new method can provide a good approximation of the three rate constants by measuring S not only near the end of the reaction but also during the quasi-steady state. This avoids the problem, requiring measuring both enzyme and substrate concentration accurately near the end of the reaction, which the former method may encounter.

4 Motivation and derivation of the key equation

In this section, we will explain how we figure out this relation and why this method is reasonable.

Table 2 Estimated values of k_1 and k_{-1} by our new method, where the exact values are $k_1 = 0.3$ and $k_{-1} = 0.1$

S	E	\tilde{k}_1	\tilde{k}_{-1}
19.38	0.02453	4.4886	4.2886
19.36	0.02454	0.3881	0.1881
19.20	0.02473	0.3005	0.1005
18.20	0.02602	0.3005	0.1005
17.20	0.02748	0.3006	0.1006
16.20	0.02904	0.3006	0.1006
15.20	0.03083	0.3007	0.1007
14.20	0.03285	0.3008	0.1008
13.20	0.03516	0.3009	0.1009
12.20	0.03781	0.3010	0.1010
11.20	0.04090	0.3012	0.1012
10.70	0.04264	0.3013	0.1013
10.20	0.04454	0.3014	0.1014
9.70	0.04661	0.3016	0.1016
8.70	0.05139	0.3019	0.1019
7.70	0.05725	0.3023	0.1023
6.70	0.06462	0.3028	0.1028
5.90	0.07204	0.3034	0.1034
5.00	0.08271	0.3044	0.1044
4.00	0.09898	0.3059	0.1059
3.00	0.12317	0.3084	0.1084
2.00	0.16292	0.3126	0.1126
1.00	0.24099	0.3192	0.1192
0.50	0.31927	0.3213	0.1213
0.30	0.36927	0.3196	0.1196
0.20	0.40213	0.3169	0.1169
0.10	0.44356	0.3117	0.1117
0.04	0.47487	0.3060	0.1060

As mentioned, the whole process of the reaction ($S(t)$, $E(t)$) can be drawn on the $S - E$ plane. Since $S(t)$ decreases when t increases, we can consider E to be a function of S . Then, $E(S)$ satisfies the following equation according to Eqs. (20) and (21):

$$dE/dS = (-k_1SE(S) + (k_{-1} + k_2)(E_0 - E(S)))/(-k_1SE(S) + k_{-1}(E_0 - E(S))) \tag{22}$$

where $S \in (0, S_0]$. To give a precise description of system consisting of Eqs. (20) and (21) or Eq. (22), we should analyze the phase field of $S - E$ plane as in [9].

Define

$$P(S, E) = -k_1SE + k_{-1}(E_0 - E),$$

$$Q(S, E) = -k_1SE + (k_{-1} + k_2)(E_0 - E).$$

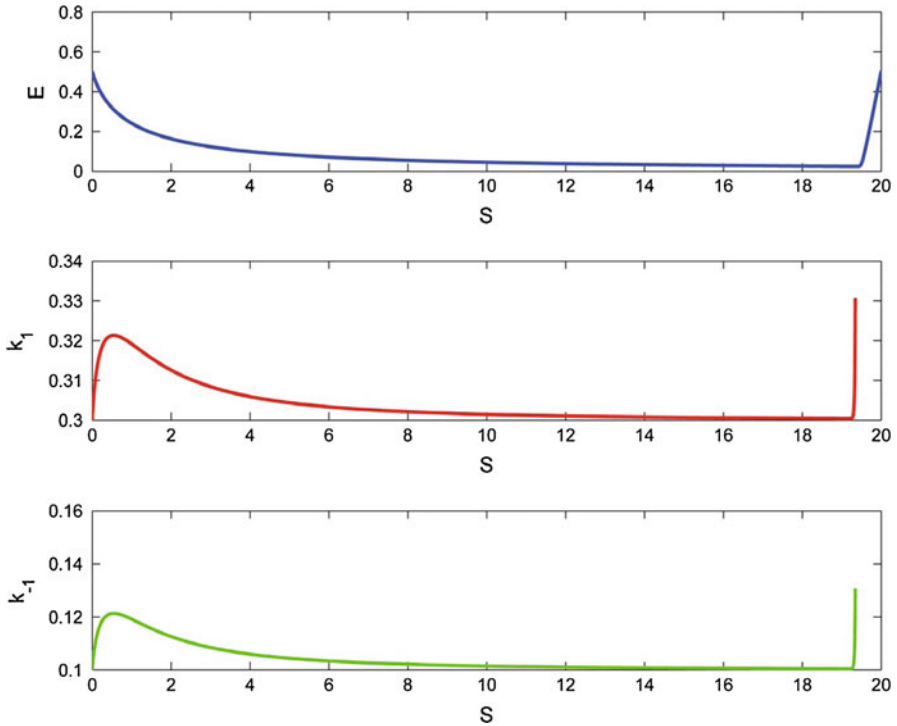


Fig. 3 Estimation given by the whole process: the first *panel* describes the whole reaction process. The next two *panels* show the corresponding estimations of k_1 and k_{-1} by the method provided in the paper. When the reaction attains its steady state, the approximation becomes very good. As S decreases, the approximation becomes worse. However, after the reaction leaves its steady state and approaches its end, the approximation becomes better and better (color figure online)

Divide the first quadrant into five regions as

$$\begin{aligned}
 L_1 &= \{(S, E) : Q(S, E) = 0, S \geq 0\}, \\
 L_2 &= \{(S, E) : P(S, E) = 0, S \geq 0\}, \\
 R_1 &= \{(S, E) : E > \tilde{E}, (S, \tilde{E}) \in L_1\}, \\
 R_2 &= \{(S, E) : \tilde{E} > E > \hat{E}, (S, \tilde{E}) \in L_1, (S, \hat{E}) \in L_2\}, \\
 R_3 &= \{(S, E) : E < \hat{E}, (S, \hat{E}) \in L_2\}.
 \end{aligned}$$

It can be proved that

$$\begin{cases} dS/dt(t) = P(S(t), E(t)) < 0 \\ dE/dt(t) = Q(S(t), E(t)) < 0 \end{cases} \quad (23)$$

in the region R_1 ,

$$\begin{cases} dS/dt(t) = P(S(t), E(t)) < 0 \\ dE/dt(t) = Q(S(t), E(t)) > 0 \end{cases} \quad (24)$$

in the region R_2 ,

$$\begin{cases} dS/dt(t) = P(S(t), E(t)) > 0 \\ dE/dt(t) = Q(S(t), E(t)) > 0 \end{cases} \tag{25}$$

in the region R_3 ,

$$\begin{cases} dS/dt(t) = P(S(t), E(t)) < 0 \\ dE/dt(t) = Q(S(t), E(t)) = 0 \end{cases} \tag{26}$$

on the curve L_1 , and

$$\begin{cases} dS/dt(t) = P(S(t), E(t)) = 0 \\ dE/dt(t) = Q(S(t), E(t)) > 0 \end{cases} \tag{27}$$

on the curve L_2 (cf. Fig. 4).

We denote $(S^*(t), E^*(t))$ as the specific solution of this system with initial condition $(S^*(0), E^*(0)) = (S_0, E_0)$ and denote $(S_*(t), E_*(t))$ as the specific solutions satisfying initial condition $(S_*(0), E_*(0)) \in L_2$ with sufficiently large $S_*(0)$. According to (27), the solution will vertically enter the region R_2 . Then, by (24), $S_*(t)$ decreases and $E_*(t)$ increases. In fact, as the proof of Lemma 3 in [9], $(S_*(t), E_*(t))$ will stay in R_2 forever and finally approaches $(0, E_0)$. In the region R_2 , the solution will go almost horizontally to the left, but at last it will approach $(0, E_0)$ with the slope $-\left(k_1 E_0 - (k_{-1} + k_2) + \sqrt{(k_1 E_0 + k_{-1} + k_2)^2 - 4k_1 k_2 E_0}\right) / 2k_{-1}$, which has been proved in [28]. Therefore, there is an inflection point on each solution curve $(S_*(t), E_*(t))$, cf. Fig. 5.

At the inflection point, $(S, E(S))$ satisfies that $d^2E/dS^2 = 0$. According to (22),

$$d^2E/dS^2 = k_1 k_2 A(S, E) / (k_1 S E(S) - k_{-1} (E_0 - E(S)))^3. \tag{28}$$

Fig. 4 The phase plane $S - E$ (color figure online)

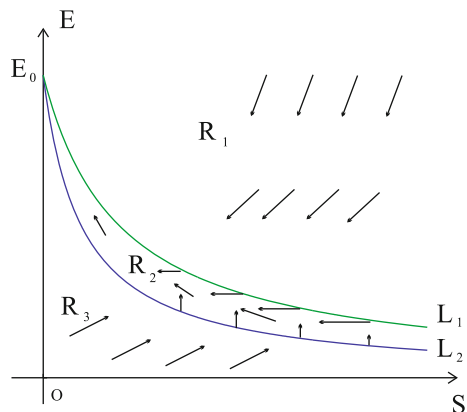


Fig. 5 Three solutions: the blue, the purple and the orange curves indicate, respectively, three solutions with the initial condition on the curve L_2 . Each of them has an inflection point (Color figure online)

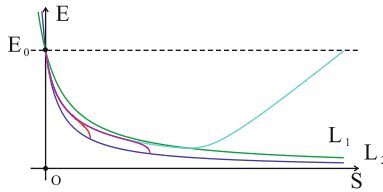
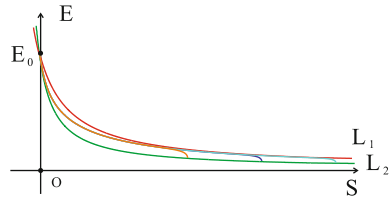
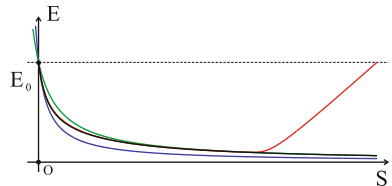


Fig. 6 Solutions with initial conditions on curve L_2 goes beneath $(S^*(t), E^*(t))$: the blue curve is the solution $(S^*(t), E^*(t))$, and the purple and red curves represent, respectively, two solutions with the initial condition on the curve L_2 . They go beneath $(S^*(t), E^*(t))$ (Color figure online)

Fig. 7 The black curve indicates a part of the curve consists of inflection points, which is under the red solution $(S^*(t), E^*(t))$ (color figure online)



where $A(S, E) = E(E_0 - E)[k_1SE - k_{-1}(E_0 - E)] + SE_0[k_1SE - (k_{-1} + k_2)(E_0 - E)]$. Therefore, the curve defined by $A(S, E) = 0$ must intersect with each solution $(S_*(t), E_*(t))$ when $S_*(0)$ is sufficiently large. As this system satisfies the existence and uniqueness condition of differential systems [31], any two different solutions will not intersect. Thus, every solution with the initial condition on the curve L_2 , that is $(S_*(t), E_*(t))$, goes beneath the solution $(S^*(t), E^*(t))$, cf. Fig. 6.

Therefore, the curve defined by $A(S, E) = 0$ has at least one part beneath the solution $(S^*(t), E^*(t))$ when S_0 is sufficient large, cf. Fig. 7.

We see that this part of the curve $A(S, E) = 0$ almost coincides with the solution $(S^*(t), E^*(t))$ after the solution passed through curve L_1 in Fig. 7. This motivates us that the part of $A(S, E) = 0$ in region R_2 approximates the solution $(S^*(t), E^*(t))$ well. Thus, we could conclude that

$$A(S^*(t), E^*(t)) \approx 0, \tag{29}$$

after the initial transient period. That is just the additional relation (14). Moreover, it also explains that during the quasi-steady state, the new method works as well.

5 Conclusion

This article provides a new relation among k_1, k_2 and k_{-1} . With this, we propose a new method to estimate all the three rate constants. Numerical results show that

this method is much better than that given in [28]. That is, this gives not only more accurate estimations than the previous one near the end of the reaction, but also very good estimations during the quasi-steady state which is beyond the applicable scope of the previous one. Actually, the latter situation is more convenient for measurement than the former. In other words, this new method not only advances the estimation accuracy, but also allows to have more choices for measurements.

The new relation $A(S, E) = 0$ comes from a mathematical observation that it is an accurate approximation of the trajectory of real reaction after the initial transient period, when S_0 is sufficiently large. To give a mathematical rigorous proof is not easy and is unnecessary in this article. We will do that in a forthcoming paper with more comprehensive study of the relationship between the curve $A(S, E) = 0$ and the real reactions.

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