Quasi-Steady State Laws in Enzyme Kinetics

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Abstract To understand how enzymes work is essential for understanding life processes. And in enzyme kinetics, an fundamental assumption is the so called Quasi-Steady State Assumption, which has the history of more than 80 years, and has been proven very fruitful in analyzing the equations of enzyme kinetics. Many experimental results and numerical results have shown the validity of the assumption. So, an important problem is if it is always true. If it is always true, then it should be a law, not only an assumption. In this paper, we prove mathematically rigorously that it is indeed always true. Hence, it is a law, and we name it Quasi-Steady State Law. Actually, more precisely, we have two Quasi-Steady State Laws. In one of them quasi-steady state means that the concentration of the enzyme-substrate complex remains approximately constant, while in the other it means that the change rate of the concentration of enzyme-substrate complex is extremely tiny.

Key words enzyme kinetics; quasi-steady state assumption; Michaelis-Menten equation; dynamical systems.

1 Introduction

Enzymes are involved in almost all the reactions of life processes and play vital role in them, so the understanding how enzymes work is essential to the understanding of life processes [1]. Enzyme kinetics, as an important branch of enzymology, is the study of the rates of chemical reactions that are catalysed by enzymes. It has attracted century-long investigation and is no less important now than it was early in the twentieth century [2]. Since enzyme kinetics is a branch of chemical kinetics, it can be characterized by some differential equations by the principles of chemical kinetics. Here we consider the simplest case that the kinetics of single substrate S and single product P reactions catalysed by enzyme E, which can be described by the following scheme

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} P + E, \tag{1}$$

where k_1 is the rate constant of formation of the enzyme-substrate complex, k_{-1} is the rate constant of dissociation of the enzyme-substrate complex and k_2 is the catalysis rate constant. In this case, based on the law of mass action the time evolution of concentrations of reactants can be determined by the following nonlinear differential equations [3]:

$$\frac{d}{dt}S = -k_1SE + k_{-1}C \tag{2}$$

$$\frac{d}{dt}E = -k_1SE + (k_{-1} + k_2)C \tag{3}$$

$$\frac{d}{dt}C = k_1SE - (k_{-1} + k_2)C \tag{4}$$

$$\frac{d}{dt}P = k_2C \tag{5}$$

with the initial condition

$$(S(0), E(0), C(0), P(0)) = (S_0, E_0, 0, 0). \tag{6}$$

Under the two conservation laws

$$E + C = E_0 \tag{7}$$

$$S + C + P = S_0, \tag{8}$$

equations (2)-(5) are equivalent to the following equations

$$\frac{d}{dt}S = -k_1SE + k_{-1}(E_0 - E) \tag{9}$$

$$\frac{d}{dt}E = -k_1SE + (k_{-1} + k_2)(E_0 - E) \tag{10}$$

with the initial condition $(S(0), E(0)) = (S_0, E_0)$. For brevity, we only consider equations (9) and (10) in this paper.

For the reason that these equations (9) and (10) cannot be integrated explicitly, Michaelis and Menten [4] proposed equilibrium assumption in 1913. They assumed that $k_{-1} >> k_2$, therefore

$$\frac{SE}{C} = \frac{k_{-1}}{k_1}. \tag{11}$$

This means that an equilibrium is established between E, S and the enzyme-substrate complex C, the slow step is the breakdown C to produce P and E. Under this assumption, the time evolution of the reactant concentrations in scheme (1) can be calculated explicitly.

In 1925, Briggs and Haldane [5] pointed out that the Michaelis assumption that an equilibrium exists between E, S and C is not always justified, and should be replaced by the assumption that C is present not necessarily at equilibrium but in a steady state under condition $S_0 >> E_0$ [6].

Here we give a more detail description of Briggs' steady state assumption by quoting the statement in Voet's famous text book [2]: With the exception of initial stage of the reaction, which is usually over within milliseconds of mixing E and S, C remains approximately constant until the substrate is nearly exhausted. Hence, the rate of synthesis of C must equal its rate of consumption over most of the course of the reaction. In other words, C maintains a steady state and can be treated as a constant value:

$$\frac{d}{dt}C = 0. \tag{12}$$

This so called steady state assumption (SSA) is a more general condition than that of equilibrium. Furthermore, it is usually referred to as quasi-steady state assumption or quasi-steady state approximation (QSSA) for the fact that

$$\frac{d}{dt}C \approx 0 \tag{13}$$

over the most course of the reaction corresponds to equation (12). By QSSA, the classic Michaelis-Menten equation

$$v_0 = \frac{V_{max}S}{K_M + S} \tag{14}$$

is obtained, where $V_{max} = k_2E_0$, $K_M = \frac{k_{-1}+k_2}{k_1}$ is the Michaelis constant and v_0 denotes the initial velocity of the reaction.

Since the work of Briggs and Haldane in 1925 [5], QSSA has become a fundamental assumption in enzyme kinetics. It has been proven very fruitful in the analysis of equations (9) and (10), yielding approximate analytical solutions and simple parameter estimation schemes [7, 8, 9, 10]. The application of QSSA in biochemical kinetics allows the reduction of a complex biochemical system with an initial fast transient into a simpler one [19]. Therefore, this kind of simplification can be used in the study of system biology such as metabolic processes and genetic regulation processes [20], for all these processes involving enzyme catalysis.

All the experimental results about enzyme kinetics so far show that the quasi-steady state assumption or the Michaelis-Menten equation provide a highly satisfactory description of enzyme kinetics for large ensembles of enzyme molecules when the concentration of substrate greatly exceeds that of enzyme [1]. At the single-molecule level, an enzyme molecule undergoes rapid thermal fluctuation and reacts stochastically with substrate molecules due to its incessant collisions with the solvent molecules [11, 12]. However, by the statistical analysis of the stochastic behave of single-molecule enzyme catalysis, Michaelis-Menten equation is still satisfied [1, 11, 13]. Therefore, QSSA is highly satisfied in all known experiments not only at assemble level of enzyme molecules, but also at single-molecule level.

Despite of the highly consistency of QSSA with known experimental results, the validity of QSSA had not been discussed until the work of Segel [14] and the work of Segel and Slemrod [15], in which a condition $E_0 << S_0 + K_M$ was given for QSSA. After that, Borghans et al [16] proposed tQSSA by a simple change of varivable and extended the parameter domain in which QSSA is valid. Schnell [3] proposed a closed form solution for the total time evolution of the reactant concentrations in scheme (1) and Schenell et al [17] found a necessary criterion that ensures the validity of rQSSA. All these previous work provided approximate analytical solutions by employing the quasi-steady state approximation and showed that these approximate solutions were very close to the numerical solutions of equations (2)-(5) with initial condition (6).

Although many strong evidence instances for the validity of QSSA have been provided from the point of experiment and approximate solutions as mentioned in the above two paragraphs, they can not ensure that this assumption is also true in undone experiments or numerical computations. Hence there is naturally a

Question : Is QSSA always true for any group of reaction rate constants or if it is only true for the reaction rate constants satisfying some conditions? If it is always true, then it would be not only an assumption, but also a law.

The answer to this question can not be given by experiments or numerical solutions of differential equations, since all these concern only finite groups of concrete reaction rate constants, not all possibilities of the constants.

Moreover, as said in [23], numerics can sometimes give seriously misleading results, hence, although the famous Lorenz attractor has been generated on computers by numerical approximations since 1963, the rigorous proof given by Tucker in 1999 is still hugely significant and become a worldwide striking event [21, 22, 23].

Thus if one can answer this question completely, the answer can be given only by a rigorous mathematical proof. There have been some significant applications of mathematics to biology, such as the pioneering application of game theory to evolution by Maynard Smith and the study of deterministic chaos into ecology done by Robert May. For brevity, we do not list all the details. Those who want to know more can refer to references [24, 25, 26, 27].

Since Poincaré's work on three-body problem [28], the qualitative theory of dynamical systems has been developed for more than 100 years. Many talented mathematicians such as Birkhoff [29], Kolmogorov [30] and Smale [31, 32] etc made their contributions to its development. In this century-long period, the qualitative theory of dynamical systems has been applied to many fields [33, 34]. But it seems that none of the published papers has tried to analyze QSSA by such a theory in the past 82 years.

Hence we have a try. Surprisingly, our trying is completely successful. So we are very glad and can't help admiring the gifted insights and excellent experimental techniques of biologists in the meanwhile.

Since we can prove that the quasi-steady state assumption is always valid under condition $S_0 >> E_0$, we call it quasi-steady state law from now on. All our proof can be well understood by those who have the undergraduate level calculus background. Moreover, the analyzing technique used in our proof should be able to apply to other more complex schemes of enzyme kinetics.

2 Quasi-Steady State Laws

In this section, we first repeat the quasi-steady state assumption as stated in the famous text book [2]: Under the physiologically common condition that substrate is in great excess over enzyme ($S_0 >> E_0$), 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.

The above description about QSSA means that $C \approx constant$ for a long time. And in the applications of QSSA, one often uses $\frac{d}{dt}C \approx 0$ instead of $C \approx constant$. But $C \approx constant$ in a period and $\frac{d}{dt}C \approx 0$ in the same period are not equivalent in general. $C \approx constant$ can not ensure $\frac{d}{dt}C \approx 0$, because $\frac{d}{dt}C$ may oscillate frequently. Conversely, $\frac{d}{dt}C \approx 0$ can not ensure $C \approx constant$ either, because C may change significantly as time goes by.

Correspondingly, we reexpress the QSSA in the following two versions. The first is, under the condition of $S_0 >> E_0$, $C \approx constant$ until the substrate is nearly exhausted with an exception of the transient initial stage of the reaction. The second is all the same to the first but $C \approx constant$ is replaced by $\frac{d}{dt}C \approx 0$.

To be more precise, we appeal to mathematical language and state them as

Quasi-Steady State Law 1: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $C(t)$ will go upwards from 0 at $t = 0$ to $E_0 - \varepsilon$ in a period less than ε, then it will stay in the interval between E_0 and $E_0 - \varepsilon$ until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

Quasi-Steady State Law 2: Given any small positive number $\varepsilon > 0$, there is a proper positive number U such that $|\frac{d}{dt}C(t)|$ will be less than ε after a fast initial period less than ε and keep this state until $S(t)/S_0 < \varepsilon$, if $S_0 > U$.

In the above two laws, ε can be any positive number which depends on the requirement of the experiments. For example, ε can be 0.1 or 0.01 or even smaller. So the statements that $C(t) \approx$ *constant* and $\frac{d}{dt}C \approx 0$ are characterized by $E_0 - \varepsilon \le C(t) \le E_0$ and $|\frac{d}{dt}C(t)| \le \varepsilon$, respectively. And the conditions that $S_0 >> E_0$ and S is nearly exhausted are described by $S_0 > U$ and $S(t)/S_0 < \varepsilon$, respectively.

No matter how small ε is, if a suitable U is chosen to make sure $S_0 > U$, then we could assure both QSSL 1 and QSSL 2 for any reaction rate constants. Of course, the criteria for choosing U is related to E_0, ε and the reaction rate constants.

3 Rigorous Proof of QSSL 1

According to section 1, the basic enzyme kinetics can be described by the equations (9) and (10), namely the equation system

$$\begin{cases} \frac{d}{dt}S = P(S,E) \\ \frac{d}{dt}E = Q(S,E) \end{cases} \tag{15}$$

where

$$\begin{aligned} P(S,E) &= -k_1 SE + k_{-1}E_0 - k_{-1}E, \\ Q(S,E) &= -k_1 SE + (k_{-1}+k_2)E_0 - (k_{-1}+k_2)E. \end{aligned}$$

Let $(S(t), E(t))$ be the solution of the system (15) with initial condition $(S(0), E(0)) = (S_0, E_0)$.

The system (15) has a unique finite equilibrium point $(0, E_0)$. Considering the linear part of system (15) at the point $(0, E_0)$, that is

$$\begin{cases} \frac{d}{dt}S = -k_1E_0S - k_{-1}(E - E_0) \\ \frac{d}{dt}E = -k_1E_0S - (k_{-1}+k_2)(E - E_0)\,, \end{cases} \tag{16}$$

the eigenvalues of this linear system are two unequal negative real numbers. Thus the equilibrium point $(0, E_0)$ of the system (15) is a stable nodal point [18].

At first, we describe some notations that will be used frequently below. Let L_1, L_2, R_1 and R_2 be the point sets (see the top panel of figure 1)

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

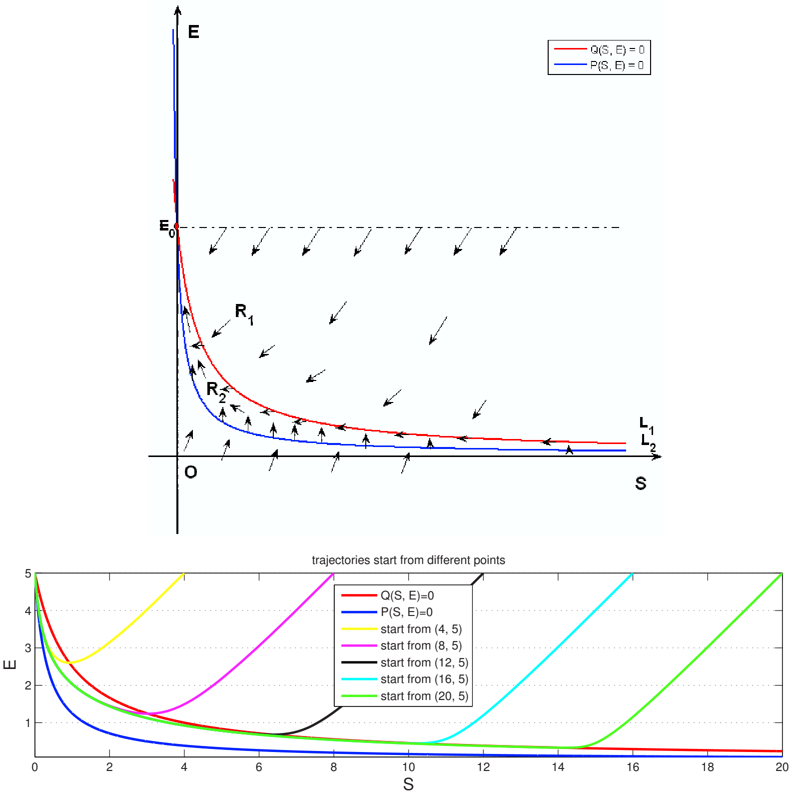

Figure 1:In the top panel, Q(S, E) = 0 and P(S, E) = 0 are two hyperbolas, which intersect each other at the point $(0, E_0)$. L_1 and L_2 are the two hyperbolas with $S \geq 0$, respectively. R_1 is the region above L_1, and R_2 is between L_1 and L_2. The arrows show the vector fields of dynamical system (15). In the low panel, we describe some trajectories of dynamical system (15) starting from different points, which are calculated by computers with parameters $k_1 = 0.3$, $k_2 = 0.2$, $k_{-1} = 0.1$.

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 the top panel of figure 1). From system (15), it is easy to deduce that

$$\left\{ \begin{array}{l} \frac{d}{dt}S = P(S, E) < 0 \\ \frac{d}{dt}E = Q(S, E) < 0 \end{array} \right. \tag{17}$$

in the region R_1,

$$\left\{ \begin{array}{l} \frac{d}{dt}S = P(S, E) < 0 \\ \frac{d}{dt}E = Q(S, E) > 0 \end{array} \right. \tag{18}$$

in the region R_2,

$$\begin{cases} \frac{d}{dt}S = P(S,E) < 0 \\ \frac{d}{dt}E = Q(S,E) = 0 \end{cases} \tag{19}$$

on the curve L_1 and

$$\begin{cases} \frac{d}{dt}S = P(S,E) = 0 \\ \frac{d}{dt}E = Q(S,E) > 0 \end{cases} \tag{20}$$

on the curve L_2.

The corresponding vector fields can be deduced by the systems of equations (17)-(20)(see the top panel of figure 1).

Lemma 1: The solution $(S(t), E(t))$ of system (15) 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 $\frac{d}{dt}E = 0$. 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, $\frac{d}{dt}E = Q(S(t_0), 0) = (k_{-1} + k_2)E_0 > 0$ at time $t = t_0$, contradicting the fact that $\frac{d}{dt}E = Q(S(t), E(t)) < -\mu$.

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 $\frac{d}{dt}E(\hat{t}) > 0$ for the initial value $E(0) = E_0$. This contradicts the fact that $\frac{d}{dt}E(t) < 0$ for $0 \leq t < T_0$. Hence the solution $(S(t), E(t))$ of system (15) will arrive at L_1 at some time $T_0 > 0$.$\square$

Lemma 2: $E(t)$ and $S(t)$ decrease monotonously and $\frac{d}{dt}E(t)$ increases 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 (15) 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 (17).

In addition,

$$\frac{d^2}{dt^2}E = -k_1 E P(S,E) - (k_1 S + k_{-1} + k_2) Q(S,E) > 0$$

for (S,E) in the region R_1. Thus, $\frac{d}{dt}E(t)$ will increase monotonously until $(S(t), E(t))$ arrives at L_1.$\square$

By inequalities (19), 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 (18)-(20) (see figure 2). 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 $(0, E_0)$.

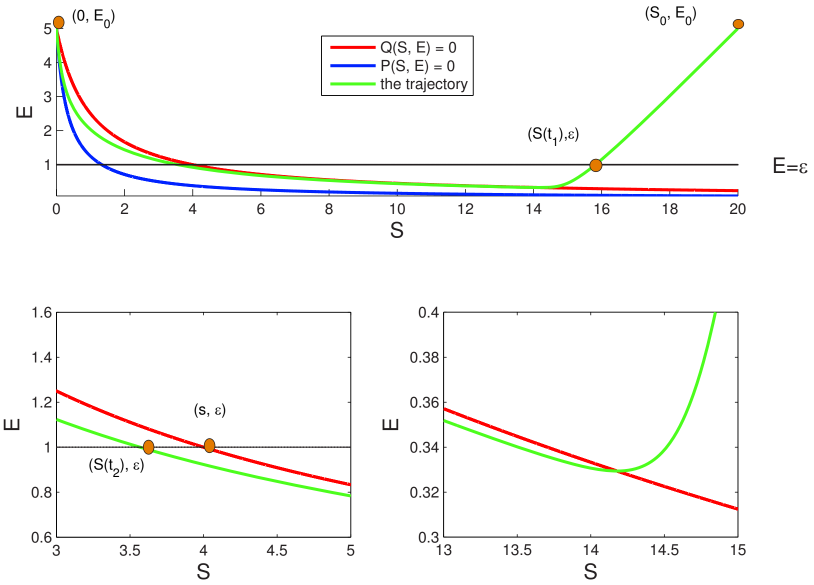

Figure 2:In this figure, we demonstrate an instance to make our proof more readable, where the parameters are chosen as $k_1 = 0.3$, $k_2 = 0.2$, $k_{-1} = 0.1$, $E_0 = 5$, $S_0 = 20$ and $\varepsilon = 1$. Q(S, E)=0 and P(S, E)=0 are two hyperbolas, which are red and blue respectively. The green curve shows the trajectory of the solution $(S(t), E(t))$ of system (15), which starts from (S_0, E_0) and goes across the hyperbola $Q(S, E) = 0$ horizontally, and then goes towards $(0, E_0)$ in the region R_2. The top panel shows the global trajectory and the corresponding hyperbolas. To see it clear, the corresponding parts of the top panel are amplified as the low left and low right panels. $(S(t_1), \varepsilon)$ and $(S(t_2), \varepsilon)$ are the intersections of the trajectory and line $E = \varepsilon$, where $t_1 < t_2$. (s, ε) is the intersection of line $E = \varepsilon$ and hyperbola $Q(S, E) = 0$. Furthermore, under the given parameters mentioned above, we have $t_1 = 0.3437376$, $t_2 = 14.102563$, $S(t_1) = 15.816376$ and $S(t_2) = 3.568946$.

In the low panel of figure 1, there are some solutions starting from different initial points. All these solutions evolve like what Lemma 3 states.

The next lemma shows that the time elapsed for $E(t)$ decreasing to any given level can be less than any given time if S_0 is chosen large enough.

Lemma 4: Given E_0 and any $\varepsilon > 0$, there exists a proper positive number U_0. If $S_0 > U_0$, $E(t)$ will decrease to a level less than ε in a period less than ε.

Proof : Choosing $\frac{E_0}{\varepsilon} > 0$, $Q(S, E) = -\frac{E_0}{\varepsilon}$ defines a hyperbola. To make sure that this hyperbola separates the point (S_0, E_0) and the curve L_1, it must restrict

$$S_0 > \frac{1}{k_1 \varepsilon}. \tag{21}$$

By this restriction, the solution $(S(t), E(t))$ of system (15) must cross the hyperbola $Q(S, E) = -\frac{E_0}{\varepsilon}$ before arriving at the curve L_1. Denote by t_ε the time to make the solution

$(S(t), E(t))$ intersecting with the curve $Q(S, E) = -\frac{E_0}{\varepsilon}$, that is $Q(S(t_\varepsilon), E(t_\varepsilon)) = -\frac{E_0}{\varepsilon}$, then $Q(S(t), E(t)) < -\frac{E_0}{\varepsilon}$ for $0 \leq t < t_\varepsilon$. Hence,

$$t_\varepsilon \leq \frac{E_0}{\frac{E_0}{\varepsilon}} = \varepsilon. \tag{22}$$

Comparing the equations (9) and (10) yields

$$\frac{dS}{dt} + k_2(E_0 - E) = \frac{dE}{dt}. \tag{23}$$

And by integrating each side of (23) from 0 to t_ε, it gets

$$\int_0^{t_\varepsilon} \frac{dE}{dt} dt = \int_0^{t_\varepsilon} [\frac{dS}{dt} + k_2(E_0 - E)] dt \leq \int_0^{t_\varepsilon} [\frac{dS}{dt} + k_2 E_0] dt.$$

So, $E(t_\varepsilon) - E_0 \leq S(t_\varepsilon) - S_0 + k_2 E_0 t_\varepsilon$. Therefore,

$$S(t_\varepsilon) \geq S_0 - k_2 E_0 t_\varepsilon + E(t_\varepsilon) - E_0 \geq S_0 - k_2 E_0 \varepsilon - E_0 \tag{24}$$

due to the inequality (22). On the hyperbola $Q(S, E) = -\frac{E_0}{\varepsilon}$, if

$$S \geq \frac{E_0}{k_1 \varepsilon^2} + \frac{(k_{-1} + k_2) E_0}{k_1 \varepsilon} - \frac{k_{-1} + k_2}{k_1}, \tag{25}$$

then

$$E \leq \varepsilon. \tag{26}$$

Now, by the inequalities (21) and (24)-(25), we have that if

$$S_0 \geq max\{\frac{E_0}{k_1 \varepsilon^2} + \frac{(k_{-1} + k_2) E_0}{k_1 \varepsilon} - \frac{k_{-1} + k_2}{k_1} + k_2 E_0 \varepsilon + E_0, \frac{1}{k_1 \varepsilon}\}, \tag{27}$$

it must have

$$E(t_\varepsilon) \leq \varepsilon. \tag{28}$$

Let

$$U_0 = max\{\frac{E_0}{k_1 \varepsilon^2} + \frac{(k_{-1} + k_2) E_0}{k_1 \varepsilon} - \frac{k_{-1} + k_2}{k_1} + k_2 E_0 \varepsilon + E_0, \frac{1}{k_1 \varepsilon}\}.$$

Then, if $S_0 > U_0$, $E(t)$ will decrease to a level less than ε in a period less than ε. □

QSSL 1: Given any $\varepsilon > 0$, there exists a proper U_1 such that if $S_0 > U_1$, $E(t)$ will decrease to a level less than ε in a period less than ε and keep the state that $E(t) \leq \varepsilon$ until $S(t)$ decreases to a level less than εS_0.

Proof : According to Lemma 4, there is a U_0 such that $E(t)$ will decrease to a level less than ε in a period less than ε, if $S_0 > U_0$. And according to Lemma 3, $E(t) = \varepsilon$ has two different solutions t_1 and t_2 where $t_1 < t_2$ (see figure 2). According to Lemma 3 and Lemma 4, the line $E = \varepsilon$ intersects the hyperbola $Q(S, E) = 0$ at the point (s, ε) such that $s > S(t_2)$ (see figure 2, box A). Thus

$$S(t_2) < s = \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon}. \tag{29}$$

Thus, a choice of

$$U_1 = \max\{U_0, \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon^2})\}$$

completes the proof.□

4 Rigorous Proof of QSSL 2

In order to prove QSSL 2, we need to consider the second order differential equation concerning C derived from the system (15). Let $V = \frac{dC}{dt}$. Then

$$\begin{aligned}
\frac{dV}{dt} &= \frac{d^2C}{dt^2} \\
&= k_1\frac{dS}{dt}E + k_1S\frac{dE}{dt} - (k_{-1}+k_2)\frac{dC}{dt} \\
&= k_1(-V-k_2C)(E_0-C) - k_1SV - (k_{-1}+k_2)V \\
&= k_1(-V-k_2C)(E_0-C) - \frac{V+(k_{-1}+k_2)C}{E_0-C}V - (k_{-1}+k_2)V
\end{aligned}$$

and $C(0)=0$, $V(0) = k_1S(0)E(0) - (k_{-1}+k_2)C(0) = k_1S_0E_0$. Thus, we get the system

$$\begin{cases} \frac{dC}{dt} = V \\ \frac{dV}{dt} = k_1(-V-k_2C)(E_0-C) - \frac{V+(k_{-1}+k_2)C}{E_0-C}V - (k_{-1}+k_2)V \end{cases} \tag{30}$$

with initial condition $(C(0), V(0)) = (0, k_1S_0E_0)$.

As considering the system (15) on the phase plane $S-E$, we first consider the vector fields on the plane $C-V$. Since $0 < E(t) \le E_0$ for any t, it appears that $0 \le C(t) < E_0$. Therefore, it is enough to consider the vector fields of the system (30) in the region $0 \le C < E_0$. Letting $\frac{dV}{dt} = 0$, it yields

$$V^2 + \Big(k_1\left(C-E_0\right)^2 + k_{-1}E_0 + k_2E_0\Big)V + Ck_1k_2\left(C-E_0\right)^2 = 0 \tag{31}$$

Regard (31) as a quadratic equation of V, then the discriminant of this equation is

$$\Delta = (k_1(C-E_0)^2 + k_{-1}E_0 + k_2E_0)^2 - 4Ck_1k_2(C-E_0)^2$$

Note that $Ck_1k_2\left(C-E_0\right)^2$ have the same sign in the region $0 < C < E_0$. According to system (15), $0 \le C(t) < E_0$ for $t \ge 0$. Thus, it is enough to consider the case $0 \le C \le E_0$. Therefore, if $\Delta > 0$ the equation (31) of V has two negative solutions when $0 < C < E_0$. When $C = E_0$, $\Delta = {E_0}^2\left(k_{-1}+k_2\right)^2 > 0$. Thus, the equation of V has two solutions. One is zero and the other is negative. It is the same when $C = 0$. Actually, when $0 \le C(t) \le E_0$

$$\begin{aligned}
\Delta(C) &= (k_1(C-E_0)^2 + k_{-1}E_0 + k_2E_0)^2 - 4Ck_1k_2(C-E_0)^2 \\
&= k_1^2(C-E_0)^4 + k_{-1}^2E_0^2 + k_2^2E_0^2 + 2k_1(C-E_0)^2k_{-1}E_0 + 2k_1(C-E_0)^2k_2E_0 \\
&\quad +2k_{-1}E_0k_2E_0 - 4Ck_1k_2(C-E_0)^2 \\
&\ge k_1^2(C-E_0)^4 + k_{-1}^2E_0^2 + k_2^2E_0^2 + 2k_1(C-E_0)^2k_{-1}E_0 + 2k_1(C-E_0)^2k_2E_0 \\
&\quad +2k_{-1}E_0k_2E_0 - 4E_0k_1k_2(C-E_0)^2 \\
&= k_1^2(C-E_0)^4 + k_{-1}^2E_0^2 + k_2^2E_0^2 + 2k_1(C-E_0)^2k_{-1}E_0 - 2k_1(C-E_0)^2k_2E_0 \\
&\quad +2k_{-1}E_0k_2E_0 \\
&= (k_1(C-E_0)^2 - k_2E_0)^2 + k_{-1}^2E_0^2 + 2k_1(C-E_0)^2k_{-1}E_0 + 2k_{-1}E_0k_2E_0 \\
&> 0
\end{aligned}$$

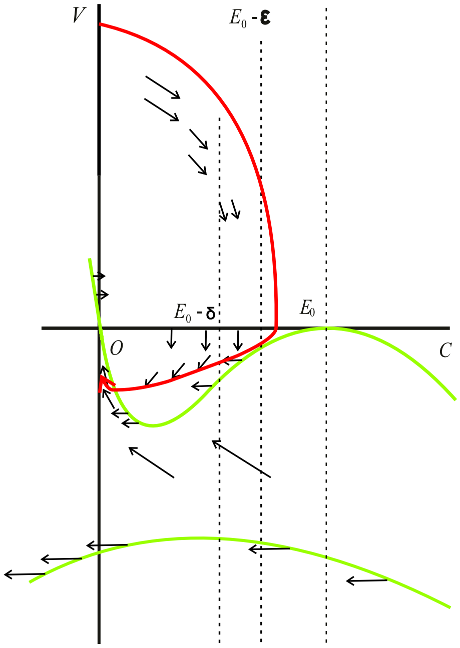

Figure 3:In this figure, the green curves indicate the shape of $\frac{dV}{dt} = 0$. The red curve indicates the solution of system (30). The arrows indicate the vector fields of the phase plane.

Since Δ is a continuous function of C, Δ is bigger than 0 in a neighborhood of the interval $[0, E_0]$. Consider the bigger one of the solutions of the equation (31) in the neighborhood of the interval $[0, E_0]$, that is

$$\begin{aligned} V_1(C) \quad = \quad & -1/2\,k_1C^2 - 1/2\,k_1{E_0}^2 + k_1E_0C - 1/2\,k_{-1}E_0 - 1/2\,k_2E_0 \\ & +1/2\,(-8\,k_1k_2{E_0}^2C + 10\,k_1k_2E_0C^2 + 2\,k_1k_{-1}E_0C^2 - 4k_1E_0^2k_{-1}C \\ & +6{k_1}^2{E_0}^2C^2 - 4{k_1}^2E_0C^3 - 4{k_1}^2{E_0}^3C + 2k_1k_{-1}E_0^3 + 2\,k_1k_2{E_0}^3 \\ & +2\,k_{-1}k_2{E_0}^2 + {k_1}^2C^4 + {k_1}^2{E_0}^4 + {k_{-1}}^2{E_0}^2 + {k_2}^2{E_0}^2 - 4\,k_1k_2C^3)^{1/2} \end{aligned}$$

To consider the approximate shape of this solution on the phase plane, the first and second derivative of V with respect to C at point $C = E_0$ should be considered. The first order derivative is

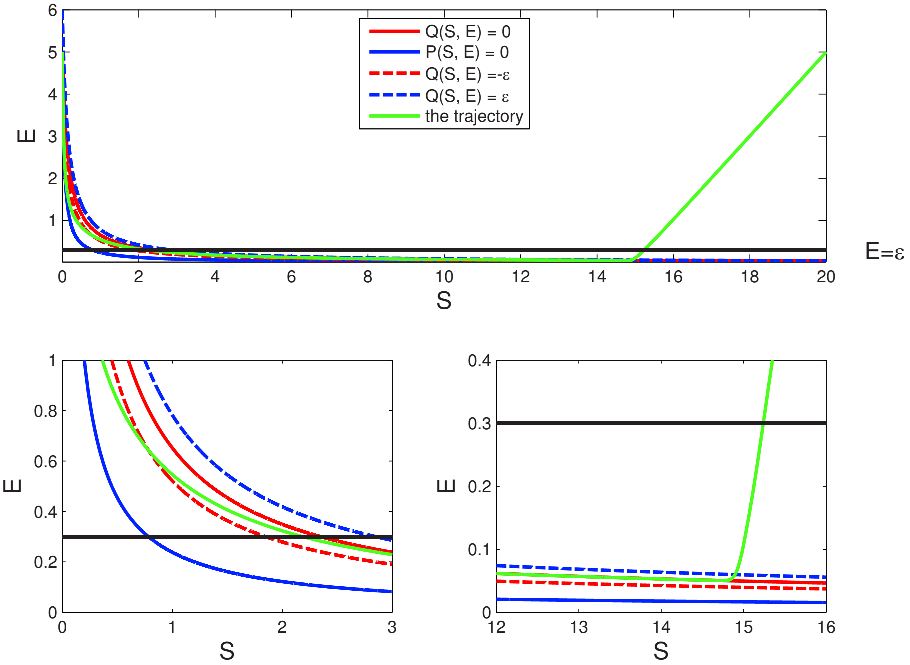

Figure 4:In this figure we choose the parameters as follows: $k_1 = 2$, $k_2 = 0.2$, $k_{-1} = 0.1$, $E_0 = 5$, $S_0 = 20$ and $\varepsilon = 0.3$. The top panel shows that the trajectory sequentially crosses line $E = \varepsilon$, hyperbola $Q(S, E) = -\varepsilon$, line $E = \varepsilon$ and hyperbola $Q(S, E) = \varepsilon$ at time 0.0892893, 0.1950208, 13.432252 and 15.345701, respectively, and the corresponding intersections are (15.238097, 0.3), (14.8948, 0.059821), (2.18855, 0.3) and (0.784883, 0.641792). To see it clear, we amplify the corresponding parts of the top panel as the low left and low right panels.

$$\begin{aligned}\frac{d}{dC}V_1(C) \quad = \quad & -k_1C + k_1E_0 + 1/4(-8\,k_1k_2{E_0}^2 + 20\,k_1k_2E_0C + 4\,k_1k_{-1}E_0C \\ & -4\,k_1k_{-1}{E_0}^2 + 12\,{k_1}^2{E_0}^2C - 12\,{k_1}^2E_0C^2 - 4\,{k_1}^2{E_0}^3 \\ & +4\,{k_1}^2C^3 - 12\,k_1k_2C^2)(-8\,k_1k_2{E_0}^2C + 10\,k_1k_2E_0C^2 \\ & +2\,k_1k_{-1}E_0C^2 - 4\,k_1k_{-1}{E_0}^2C + 6\,{k_1}^2{E_0}^2C^2 - 4\,{k_1}^2E_0C^3 \\ & -4\,{k_1}^2{E_0}^3C + 2\,k_1k_{-1}{E_0}^3 + 2\,k_1k_2{E_0}^3 + 2\,k_{-1}k_2{E_0}^2 + {k_1}^2C^4 \\ & +{k_1}^2{E_0}^4 + {k_{-1}}^2{E_0}^2 + {k_2}^2{E_0}^2 - 4\,k_1k_2C^3)^{-1/2}\end{aligned}$$

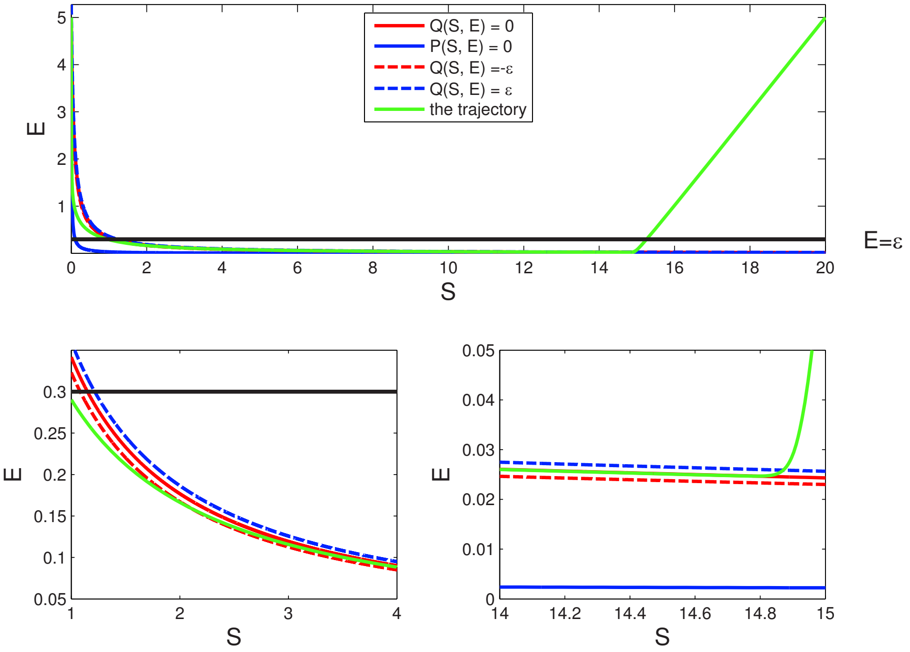

Figure 5:In this figure we choose the parameters as follows: $k_1 = 15$, $k_2 = 1$, $k_{-1} = 0.1$, $E_0 = 5$, $S_0 = 20$ and $\varepsilon = 0.3$. The top panel shows that the trajectory sequentially crosses line $E = \varepsilon$, hyperbola $Q(S, E) = -\varepsilon$, hyperbola $Q(S, E) = \varepsilon$ and line $E = \varepsilon$ at time 0.011592, 0.035041, 2.642929 and 2.913193, respectively, and the corresponding intersections are (15.26009, 0.3), (14.870496, 0.025875), (2.107647, 0.15895) and (0.956045, 0.3). To see it clear, we amplify the corresponding parts of the top panel as the low left and low right panels.

It is equal to 0 at $C = E_0$. The second order derivative is

$$\begin{aligned}
\frac{d^2}{dC^2}V_1(C) \quad = \quad & -k_1 - 1/8((-8\,k_1k_2{E_0}^2 + 20\,k_1k_2E_0C + 4\,k_1k_{-1}E_0C \\
& -4\,k_1k_{-1}{E_0}^2 + 12\,{k_1}^2{E_0}^2C - 12\,{k_1}^2E_0C^2 - 4\,{k_1}^2{E_0}^3 + 4\,{k_1}^2C^3 \\
& -12\,k_1k_2C^2)^2)((-8\,k_1k_2{E_0}^2C + 10\,k_1k_2E_0C^2 + 2\,k_1k_{-1}E_0C^2 \\
& -4\,k_1k_{-1}{E_0}^2C + 6\,{k_1}^2{E_0}^2C^2 - 4\,{k_1}^2E_0C^3 - 4\,{k_1}^2{E_0}^3C \\
& +2\,k_1k_{-1}{E_0}^3 + 2\,k_1k_2{E_0}^3 + 2\,k_{-1}k_2{E_0}^2 + {k_1}^2C^4 + {k_1}^2{E_0}^4 \\
& +{k_{-1}}^2{E_0}^2 + {k_2}^2{E_0}^2 - 4\,k_1k_2C^3)^{3/2})^{-1} + 1/4(20\,k_1k_2E_0 + 4\,k_1k_{-1}E_0 \\
& +12\,{k_1}^2{E_0}^2 - 24\,{k_1}^2E_0C + 12\,{k_1}^2C^2 - 24\,k_1k_2C)(-8\,k_1k_2{E_0}^2C \\
& +10\,k_1k_2E_0C^2 + 2\,k_1k_{-1}E_0C^2 - 4\,k_1k_{-1}{E_0}^2C + 6\,{k_1}^2{E_0}^2C^2 \\
& -4\,{k_1}^2E_0C^3 - 4\,{k_1}^2{E_0}^3C + 2\,k_1k_{-1}{E_0}^3 + 2\,k_1k_2{E_0}^3 + 2\,k_{-1}k_2{E_0}^2 \\
& +{k_1}^2C^4 + {k_1}^2{E_0}^4 + {k_{-1}}^2{E_0}^2 + {k_2}^2{E_0}^2 - 4\,k_1k_2C^3)^{-1}
\end{aligned}$$

It is equal to $-2\,k_1k_2\,(k_{-1}+k_2)$ at $C = E_0$. Thus, $V_1(C)$ is concave in a neighborhood of $C = E_0$. Namely, there is $\delta > 0$ such that $V_1(C)$ is concave when $E_0 - \delta < C < E_0 + \delta$ and $C = E_0$ is the critical point. Thus, $V_1(C)$ increases with respect to C on the interval $(E_0 - \delta, E_0]$ and $V_1(C) < 0$ for $C \in (E_0 - \delta, E_0)$.

When $V > 0$ and $0 \le C < E_0$, $\frac{dV}{dt}$ is less than 0. On the curve given by the equation (31), $\frac{dV}{dt} = 0$ and $\frac{dC}{dt} = V < 0$. In the interval $(0, E_0)$ of C axis, $\frac{dC}{dt} = 0$ and $\frac{dV}{dt} < 0$. Thus, the vector fields in the region $V > 0$ and $0 \le C < E_0$ and in the region near the point $(E_0, 0)$ are obtained.

Choose $\varepsilon < \delta$. As discussed in the proof of QSSL 1, $C(t) = E_0 - E(t)$ is bigger than $E_0 - \varepsilon$ when $t_1 < t < t_2$ if $S_0 > U_0$. In the phase plane $C - V$, the integral curve can not intersect the curve $\frac{dV}{dt} = 0$ while $E_0 - \delta < C \le E_0$ (see figure 3). Therefore, $\frac{dV}{dt} < 0$ while $t < t_2$. That is to say

$$\frac{d^2E}{dt^2} > 0 \tag{32}$$

when $t < t_2$.

Lemma 5: Given E_0 and $\varepsilon > 0$, there exists U_2. If $S_0 > U_2$, $\mid \frac{d}{dt}E(t) \mid$ will decrease to a level less than ε in a period less than ε.

Proof : Choosing $\frac{2E_0}{\varepsilon} > 0$, $Q(S, E) = -\frac{2E_0}{\varepsilon}$ defines a hyperbola. To make sure that this hyperbola separates the point (S_0, E_0) and the curve L_1, we restrict

$$S_0 > \frac{2}{k_1\varepsilon}. \tag{33}$$

By this restriction, the solution $(S(t), E(t))$ of system (15) must cross the hyperbola $Q(S, E) = -\frac{2E_0}{\varepsilon}$ before arriving at the curve L_1. Denote by t'_ε the time to make the solution $(S(t), E(t))$ intersecting with the curve $Q(S, E) = -\frac{2E_0}{\varepsilon}$, that is $Q(S(t'_\varepsilon), E(t'_\varepsilon)) = -\frac{2E_0}{\varepsilon}$, then $Q(S(t), E(t)) < -\frac{2E_0}{\varepsilon}$ for $0 \le t < t'_\varepsilon$. Hence,

$$t'_\varepsilon \le \frac{E_0}{\frac{2E_0}{\varepsilon}} = \frac{\varepsilon}{2} \tag{34}$$

By integrating each side of (23) from 0 to t'_ε, it gets

$$\int_0^{t'_\varepsilon} \frac{dE}{dt}dt = \int_0^{t'_\varepsilon} [\frac{dS}{dt} + k_2(E_0 - E)]dt \le \int_0^{t'_\varepsilon} [\frac{dS}{dt} + k_2E_0]dt.$$

So, $E(t'_\varepsilon) - E_0 \le S(t'_\varepsilon) - S_0 + k_2E_0t'_\varepsilon$. Therefore,

$$S(t'_\varepsilon) \ge S_0 - k_2E_0\frac{\varepsilon}{2} + E(t'_\varepsilon) - E_0 \ge S_0 - k_2E_0\frac{\varepsilon}{2} - E_0 \tag{35}$$

due to the inequality (34). On the hyperbola $Q(S, E) = -\frac{2E_0}{\varepsilon}$, if

$$S \ge \frac{4E_0}{k_1\varepsilon^3} + \frac{2(k_{-1}+k_2)E_0}{k_1\varepsilon^2} - \frac{k_{-1}+k_2}{k_1}, \tag{36}$$

then

$$E \le \frac{\varepsilon^2}{2}. \tag{37}$$

Now, by the inequalities (33) and (35)-(36), we have that if

$$S_0 \geq max\{\frac{4E_0}{k_1\varepsilon^3} + \frac{2(k_{-1}+k_2)E_0}{k_1\varepsilon^2} - \frac{k_{-1}+k_2}{k_1} + k_2E_0\frac{\varepsilon}{2} + E_0, \frac{2}{k_1\varepsilon}\}, \tag{38}$$

it must have

$$E(t_\varepsilon) \leq \frac{\varepsilon^2}{2}. \tag{39}$$

Let

$$U_2 = max\{\frac{4E_0}{k_1\varepsilon^3} + \frac{2(k_{-1}+k_2)E_0}{k_1\varepsilon^2} - \frac{k_{-1}+k_2}{k_1} + k_2E_0\frac{\varepsilon}{2} + E_0, \frac{2}{k_1\varepsilon}\}.$$

Then, if $S_0 > U_2$, $E(t)$ will decrease to a level less than $\frac{\varepsilon^2}{2}$ in a period less than $\frac{\varepsilon}{2}$.

So far, the only thing left is to prove that $\mid \frac{d}{dt}E(t) \mid$ will go down to a level less than ε in a period less than ε. By the inequality (39), $\frac{d}{dt}E(t) < -\varepsilon$ can not last longer than $\frac{\varepsilon^2}{2}/\varepsilon = \frac{\varepsilon}{2}$ from t_ε until $\mid \frac{d}{dt}E(t) \mid \leq \varepsilon$. Therefore, $\mid \frac{d}{dt}E(t) \mid$ will decrease to the level less than ε in a period less than ε.□

Now it is easy to prove

QSSL 2: Given any $\varepsilon > 0$, there exists a proper U_3 such that if $S_0 > U_3$, $\mid \frac{dE}{dt}(t) \mid$ will decrease to a level less than ε in a period less than ε and keep the state that $\mid \frac{dE(t)}{dt} \mid \leq \varepsilon$ until $S(t)$ decreases to a level less than εS_0.

Proof : According to Lemma 5, if $S_0 > U_2$, $\mid \frac{dE}{dt}(t) \mid$ will decrease to a level less than ε in a period less than ε. Further more, according to Lemma 4 $S(t_2) < s < \frac{(k_1+k_2)(E_0-\varepsilon)}{k_1\varepsilon}$, if $S_0 > U_0$.

If $\frac{dE}{dt}(t_2) \leq \varepsilon$ (see figure 4 for instance), then $\frac{dE}{dt}(t) \leq \varepsilon$ for all $t \leq t_2$ and $\mid \frac{dE}{dt}(t) \mid \leq \varepsilon$ is kept in the time interval between ε and t_2 by (32). Hence, a choice of

$$U_3 = max\{\frac{(k_1+k_2)(E_0-\varepsilon)}{k_1\varepsilon^2}, U_0, U_2\}$$

completes the proof.

If $\frac{dE}{dt}(t_2) > \varepsilon$ (see figure 5 for instance), then $\frac{dE}{dt}(t_2 - 1) \leq \varepsilon$.

By integrating each side of the equation (23) over the interval $[t_1, t_2]$,

$$\int_{t_1}^{t_2} \frac{dE}{dt}dt = \int_{t_1}^{t_2} [\frac{dS}{dt} + k_2(E_0 - E(t))]dt.$$

In virtue of this,

$$\begin{aligned} E(t_2) - E(t_1) &= S(t_2) - S(t_1) + \int_{t_1}^{t_2} k_2(E_0 - E(t))dt \\ &\leq S(t_2) - S(t_1) + k_2E_0(t_2 - t_1). \end{aligned}$$

Since $E(t_1) = E(t_2) = \varepsilon$,

$$t_2 - t_1 \geq \frac{S(t_1) - S(t_2)}{k_2E_0}. \tag{40}$$

According to Lemma 4, if $S_0 > U_0$, $E(t)$ will be less than ε at time t_ε, that means $t_\varepsilon > t_1$. Thus

$$S(t_1) > S(t_\varepsilon) \geq S_0 - k_2 E_0 \varepsilon - E_0. \tag{41}$$

From inequalities (40),(41) and (29), it is obtained that

$$t_2 - t_1 > (S_0 - k_2 E_0 \varepsilon - E_0 - \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon})/(k_2 E_0). \tag{42}$$

Therefore, $E(t) \leq \varepsilon$ lasts for a period more than

$$(S_0 - k_2 E_0 \varepsilon - E_0 - \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon})/(k_2 E_0),$$

when $S_0 > U_0$. Thus, if

$$S_0 > max\{(1 + \varepsilon) k_2 E_0 + \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon} + E_0 + k_2 E_0 \varepsilon, U_0\}$$

$E(t) \leq \varepsilon$ lasts for a period more than $(1+\varepsilon)$. Then $t_2 > 1 + t_1 + \varepsilon$.

Hence, $\frac{dE}{dt}(t) \leq \varepsilon$ for all $t \leq t_2 - 1$ and $\mid \frac{dE}{dt}(t) \mid \leq \varepsilon$ is kept in the time interval between ε and $t_2 - 1$ by (32).

By integrating each side of equation (23) from $t_2 - 1$ to t_2, it yields

$$\int_{t_2-1}^{t_2} \frac{dE}{dt} dt = \int_{t_2-1}^{t_2} [\frac{dS}{dt} + k_2(E_0 - E(t))] dt.$$

Thus,

$$\begin{aligned} E(t_2) - E(t_2 - 1) &= S(t_2) - S(t_2 - 1) + \int_{t_2-1}^{t_2} k_2(E_0 - E(t)) dt \\ &< S(t_2) - S(t_2 - 1) + k_2 E_0. \end{aligned}$$

Rearranging the terms in the above inequality yields

$$\begin{aligned} S(t_2 - 1) &< S(t_2) + k_2 E_0 + E(t_2 - 1) - E(t_2) \\ &< s + k_2 E_0 \\ &= \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon} + k_2 E_0. \end{aligned}$$

So, a choice of

$$U_3 = max\{(1+\varepsilon) k_2 E_0 + \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon} + E_0 + k_2 E_0 \varepsilon, \frac{(k_{-1} + k_2)(E_0 - \varepsilon)}{k_1 \varepsilon^2} + \frac{k_2 E_0}{\varepsilon}, U_0, U_2\}$$

completes the proof.

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