A Positive Real Eigenvalue Condition for the Determination of Unstable Steady States in Chemical Reaction Networks

An-Chong Chao, Hsing-Ya Li, Guo-Syong Chuang, and Pang-Yen Ho

Department of Chemical Engineering, National United University, Miao-Li 360, Taiwan R. O. C.

Reprint requests to A.-C. C.; Fax: +886-37-332397; E-mail: an-chong@nuu.edu.tw

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The interesting dynamical behaviours exhibiting in chemical reaction systems, such as multiple steady states and undamped oscillations, often result from unstable steady states. A positive real eigenvalue condition is proposed which gives a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue in general isothermal reaction networks. Formulas are developed to construct an unstable steady state and a set of positive rate constants. The applications are illustrated by three examples. Two give rise to oscillations and one admits multiple steady states.

Key words: Chemical Reaction Network; Unstable Steady State; Oscillation; Positive Real Eigenvalue; Multiple Steady States.

1. Introduction

Some interesting dynamic phenomena in chemical reactors, such as unstable steady states, undamped oscillations, and multiple steady states, have been shown to occur under both isothermal and non-isothermal conditions. There are a growing number of experimental systems in which the interesting behaviour is exhibited in isothermal reactors [1 – 5] and biological systems [6 – 8]. This is to say that instabilities derive not from thermal effects but rather from the intricacy of chemistry itself. The identification of such reaction mechanisms is important, since it not only helps experimentalists to determine reaction mechanisms but also helps engineers to design more efficient and safer reaction processes.

Some results of the chemical reaction network theory have been based upon classification of reaction networks by means of a nonnegative integer index called the deficiency. Work of Horn [9], Horn and Jackson [10], and Feinberg [11] led to the deficiency zero theorem: for any deficiency zero network, no matter what the positive rate constants are, there is at most one positive steady state; that steady state is stable and there does not exist any cyclic solution. However, the stable property of deficiency zero networks is not shared in a general way by networks of higher deficiency. The deficiency one theorem [12] and the deficiency one algorithm [13] provide means to distinguish between those (deficiency one) mechanisms which can exhibit multiple steady states and those which cannot. Besides these deficiency-oriented theories, the subnetwork analysis [14, 15] and the SCL (species-complex-linkage) graph method [16] can also be applied to determine the possibility of multiple steady states. The stoichiometric network analysis developed by Clarke [17] is a powerful tool to study the stability of complex reaction networks. Based on the stoichiometric network analysis, conditions for the occurrence of a Hopf bifurcation in reaction networks are proposed in [18, 19].

The interesting dynamical behaviours often occur because of the existence of unstable steady states. A perturbation of an unstable steady state may lead to other stable steady states, undamped oscillations, and so on. The stability of a steady state can be analyzed by linearizing the corresponding systems of ordinary differential equations and examining the eigenvalues of the Jacobian [20]. If there exists a positive real part of eigenvalues, the steady state is unstable. If all the real parts of eigenvalues are negative, the steady state is stable. More subtle nonlinear stability analysis is needed for zero eigenvalue situations [20]. In this paper, we study the possibility of the existence of an unstable steady state having a positive real eigenvalue for chemical reaction networks. (Assumptions are that reacting
2. Basic Theoretical Considerations

Terminology is introduced by examples and Table 1. (Formal definitions can be found in [12, 13].) Consider the \"Brusselator\" studied by Glansdorff and Prigogine [21]. Stripping away species assumed to have constant concentrations, it is

\[ 0 \rightleftharpoons A_1 \rightarrow A_2, \]
\[ 2A_1 + A_2 \rightarrow 3A_1. \]  

We will use the symbol \( N \) to denote the number of species in a network under consideration. Thus, for network (1) \( N = 2 \). By \( \mathbb{R}^N \) we shall mean the usual vector space of \( N \)-tuples of real numbers. The standard basis for \( \mathbb{R}^N \) will be denoted \( \{ A_1, A_2, \ldots, A_N \} \).

The complexes of a network are the objects that appear before and after reaction arrows. Thus the set of complexes for network (1) is \( \{ 0, A_1, A_2, 2A_1 + A_2, 3A_1 \} \). Given a network with \( N \) species, we shall associate with each complex a vector in \( \mathbb{R}^N \). Consider network (1). With complex 0 (zero) we associate the complex vector 0 in \( \mathbb{R}^2 \); with complex 2A_1 + A_2 we associate the complex vector \( 2A_1 + A_2 \); and so on. [Note that 0 (zero) is a complex but not a species. Its physical meaning is \"surroundings\". The reaction 0 \( \rightarrow \) A_1 indicates that species A_1 flows from surroundings into the system; the reaction A_1 \( \rightarrow \) indicates that species A_1 flows from the system into surroundings.]

We shall write \( y_i \rightarrow y_j \) (or the abbreviation \( i \rightarrow j \)) to indicate the reaction whereby the complex \( y_i \) acts to the complex \( y_j \). We denote the set of reactions in a network by the symbol \( R \). Thus the set of reactions in network (1) is \( R = \{ 0 \rightarrow A_1, A_1 \rightarrow 0, A_1 \rightarrow A_2, 2A_1 + A_2 \rightarrow 3A_1 \} \). We reserve the symbol \( r \) for the number of distinct reactions in a network. For network (1), \( r = 4 \). A reaction \( y_i \rightarrow y_j \) is said to be reversible if its reverse reaction \( y_j \rightarrow y_i \) is also in the network; otherwise, the reaction \( y_i \rightarrow y_j \) is irreversible. We shall call reversible reactions \( y_i \rightleftharpoons y_j \) a reversible reaction pair. The symbol \( p \) indicates the number of distinct irreversible reaction pairs in a network. The number of irreversible reactions in a network is \( r - 2p \). For network (1), there are one \( (p = 1) \) reversible reaction pair and two \( (r - 2p = 4 - 2 = 2) \) irreversible reactions.

With each reaction of the network we associate a reaction vector in \( \mathbb{R}^N \) obtained by subtracting the \"reactant\" complex vector \( y_i \) from the \"product\" complex vector \( y_j \), i.e. \( y_j - y_i \). Consider network (1). For reaction 0 \( \rightarrow A_1 \), the corresponding reaction vector in \( \mathbb{R}^2 \) is \( A_1 - 0 = A_1 \). The set of reaction vectors for network (1) is \( \{ A_1, A_2 - A_1, A_1 - A_2 \} \).

We shall say that a reaction network has rank \( s \), if there exists a linearly independent set of \( s \) reaction vectors for the network and there exists no linearly independent set of \( s + 1 \) reaction vectors. The set of two reaction vectors \( \{ A_1, A_2 - A_1 \} \) for network (1) is linearly independent, but any set of three reaction vectors for network (1) is linearly dependent. Thus, the rank of systems are under isothermal condition and follow the mass action law.) Based on linear algebra, a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue in general reaction networks is proposed. It forms a system of inequalities and equations. If a set of solutions exists, the corresponding mass action differential equations for the network under study exhibit an unstable steady state for certain positive rate constants. Otherwise, the network admits no positive-real-eigenvalue steady states no matter what positive rate constants the system might have. The formulas to compute an unstable steady state and a set of positive rate constants are also developed. Several reaction networks are studied to illustrate the applications. Undamped oscillations and multiple steady states are presented.

Table 1. Used symbols and nomenclature.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>( A_1, A_2, \ldots )</td>
<td>chemical species</td>
</tr>
<tr>
<td>( c_1, c_2, \ldots )</td>
<td>molar concentrations for species ( A_1, A_2, \ldots ), mol/l</td>
</tr>
<tr>
<td>( e^c, e^p )</td>
<td>composition vectors at stable steady states, mol/l</td>
</tr>
<tr>
<td>( d^{(k)} )</td>
<td>a set of spanning-subnetwork vectors in ( \mathbb{R}^N )</td>
</tr>
<tr>
<td>( k_{i\rightarrow j} )</td>
<td>reaction vector set in a spanning subnetwork</td>
</tr>
<tr>
<td>( L )</td>
<td>rate constant for reaction ( i \rightarrow j ), (mol/l)(^{1-1}) reaction order ( -s-1 ) flow rate, s(^{-1} )</td>
</tr>
<tr>
<td>( \mathbb{R}^N )</td>
<td>vector space for ( r )-tuples of ( (i \rightarrow j) ) of real numbers</td>
</tr>
<tr>
<td>( \mathbb{R}^r )</td>
<td>number of distinct reactions in a network</td>
</tr>
<tr>
<td>( \mathbb{R}^N )</td>
<td>stoichiometric subspace for a network</td>
</tr>
<tr>
<td>( s )</td>
<td>number of ranks in a network</td>
</tr>
<tr>
<td>( t )</td>
<td>time, s</td>
</tr>
<tr>
<td>( y_i, y_j )</td>
<td>complex vectors</td>
</tr>
<tr>
<td>( \alpha_i )</td>
<td>variables used in the positive real eigenvalue condition</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>the nonzero real eigenvector</td>
</tr>
<tr>
<td>( \kappa_{i\rightarrow j} )</td>
<td>variables defined in (7c)–(7g)</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>a positive real eigenvector</td>
</tr>
<tr>
<td>( \omega_{i\rightarrow j} )</td>
<td>variables defined in (7a)</td>
</tr>
<tr>
<td>( \bar{\omega}_{i\rightarrow j} )</td>
<td>the standard basis for vector space ( \mathbb{R}^N )</td>
</tr>
<tr>
<td>( \xi )</td>
<td>used in the positive real eigenvalue condition</td>
</tr>
</tbody>
</table>
network (1) is two, and for it we write \( s = 2 \).

The stoichiometric subspace for a network is the span of its reaction vectors. We shall reserve the symbol \( S_1 \) to designate the stoichiometric subspace for a network. It is clear that a stoichiometric subspace is a linear subspace of \( \mathbb{R}^N \). The dimension of a stoichiometric subspace is equal to the rank \( s \) of its network. The stoichiometric subspace for network (1) is the span generated by the reaction vectors \( \{A_1, A_2 - A_1\} \), which is \( \mathbb{R}^2 \).

We shall say that a vector \( \phi = [\phi_1, \ldots, \phi_N] \in \mathbb{R}^N \) is sign compatible with (s. c. w.) a vector \( \sigma = [\sigma_1, \ldots, \sigma_N] \in \mathbb{R}^N \), if \( \sigma_L = \text{sign} \phi_L \), \( L = 1, 2, \ldots, N \). This means that \( \sigma_L \) is positive, if \( \phi_L \) is positive, \( \sigma_L \) is negative, if \( \phi_L \) is negative, and \( \sigma_L \) is zero, if \( \phi_L \) is zero.

Suppose a network under consideration has \( r \) distinct reactions with \( p \) reversible reaction pairs and \( r - 2p \) irreversible reactions. A spanning subnetwork of the network under consideration is a network consisting of all the \( r - 2p \) irreversible reactions and one and only one reaction of each reversible reaction pair. There are \( r - p = (r - 2p) + p \) reactions in a spanning subnetwork. We shall reserve the symbol \( F \) to denote the set of reactions in a spanning subnetwork. The network

\[
0 \rightarrow A_1 \rightarrow A_2, 2A_1 + A_2 \rightarrow 3A_1
\]

is a spanning subnetwork of network (1), since it consists of the two irreversible reactions and one reaction of the reversible reaction pair in network (1). For it we write \( F = \{0 \rightarrow A_1, A_1 \rightarrow A_2, 2A_1 + A_2 \rightarrow 3A_1\} \).

A network having one or more than one reversible reaction pairs \( (p \geq 1) \) will contain more than one spanning subnetwork. In what follows it will be understood that we have chosen to work with a fixed (but arbitrary) spanning subnetwork in each case under study. For a chosen spanning subnetwork, we shall construct a set of corresponding spanning-subnetwork vectors \( \{d^{(1)}, d^{(2)}, \ldots, d^{(r-p-s)}\} \) in a vector space \( \mathbb{R}^r \). Let \( \{\omega_{i-j} : i \rightarrow j \in F\} \) be the standard basis for \( \mathbb{R}^r \).

These spanning-subnetwork vectors are \( r - p - s \) linearly independent (nonzero) solutions to the vector equation

\[
\sum_{i-j \in F} d^{(L)}_{i-j} (y_j - y_i) = 0, \quad L = 1, 2, \ldots, r - p - s. \quad (3a)
\]

(A solution is a family of numbers \( \{d^{(L)}_{i-j} : i \rightarrow j \in F\}. \)

Then, \( d^{(L)}, L = 1, 2, \ldots, r - p - s \), are vectors defined in the following way:

\[
d^{(L)} = \sum_{i-j \in F} d^{(L)}_{i-j} \omega_{i-j}. \quad (3b)
\]

Consider network (1). For an arbitrary spanning subnetwork, there is one \( (r - p - s = 4 - 1 - 2 = 1) \) spanning-subnetwork vector \( d^{(1)} \) in \( \mathbb{R}^r \). Let network (2) be the chosen spanning subnetwork. By (3a) we have

\[
d^{(1)}_{0-A_1} (A_1 - 0) + d^{(1)}_{A_1-A_2} (A_2 - A_1) + d^{(1)}_{2A_1+A_2-3A_1} (3A_1 - 2A_1 - A_2) = 0. \quad (3c)
\]

A set of nonzero linearly independent solutions to the above equation is

\[
d^{(1)}_{0-A_1} = 0, \quad d^{(1)}_{A_1-A_2} = 1, \quad d^{(1)}_{2A_1+A_2-3A_1} = 1. \quad (4)
\]

Let \( e = [c_1, c_2, \ldots, c_N] \) be a composition vector in \( \mathbb{P}^N \) (nonnegative orthant of \( \mathbb{R}^N \)) for species \( A_L, L = 1, 2, \ldots, N \). In general, the set of isothermal mass action differential equations describing the behaviour of a reaction network can be written as

\[
\frac{de}{dt} = f(e) = (f_1(e), \ldots, f_N(e)) = \sum_{i-j \in R} k_{i-j} \left( \prod_{L=1}^{N} c_L^{y_{i_L}} \right) (y_j - y_i), \quad (5a)
\]

where \( y_i \) and \( y_j \) denote, respectively, the reactant and product complex, \( y_{i_L} \) denotes the stoichiometric coefficient of species \( A_L \) in the reactant complex \( y_i \), and \( k_{i-j} \) denotes the rate constant for reaction \( i \rightarrow j \). The mass action differential equations for network (1) are

\[
\frac{dc_1}{dt} = k_{0-A_1} - k_{A_1-0} c_1 - k_{A_1-A_2} c_1 + k_{2A_1+A_2-3A_1} c_1^2 c_2, \quad (5b)
\]

By a steady state of a reaction system we shall mean a composition \( c^* \) satisfying \( 0 = f(c^*) \). By a positive steady state we shall mean a steady state at which all species concentrations are positive. Only positive steady states are considered in this article.

For each composition \( e \in \mathbb{P}^N \) the right-hand equation of differential (5) is a linear combination of all reaction vectors in its network. This is to say that \( de/dt \)
always lies in the stoichiometric subspace $S_t$ of the network under consideration. Moreover, a composition $c_1$ can evolve to a composition $c_2$ only, if such a composition change $c_2 - c_1$ lies in $S_t$ [9]. Suppose $\gamma = c - c^*$ be a vector of composition change around a positive steady state $c^*$. Then $\gamma$ is restricted to $S_t$. Any vector $\gamma \in S_t$ of a given network can be represented by reaction vectors in the reaction set $F$ for an arbitrary chosen spanning subnetwork:

$$\gamma = [\gamma_1, \gamma_2, \ldots, \gamma_N] = \sum_{i-j \in F} \gamma_{i-j} (y_j - y_i).$$

3. Results and Discussion

A positive real eigenvalue condition is proposed below. It provides a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue with its eigenvector lying in the stoichiometric subspace for general reaction networks with any deficiency. For a given reaction network, we choose an arbitrary spanning subnetwork and construct its spanning-subnetwork vectors. Following the condition proposed below, a system of equations and inequalities is constructed. We then solve the system. If a set of qualified solutions exists, there is a set of positive rate constants such that the corresponding isothermal mass action differential equations for the given network admit an unstable steady state with a positive real eigenvalue. Otherwise, no matter what positive rate constants the system might have, the differential equations cannot exhibit any positive-real-eigenvalue steady state.

The logic behind the theorem is explained in the following. We derive the steady state equation (5a) and its derivative equation. A linear transformation is set up to describe these two equations by vectors such that these vectors lie in the kernel of the linear transformation. A basis is constructed for the kernel of the linear transformation and the two vectors are represented by the basis. From these equations we construct the conditions such that the steady state is nonnegative and one of its eigenvalues is a real positive number. The proof of the condition is shown in the supporting information.

3.1. Positive Real Eigenvalue Condition

Consider an $N$-species reaction network with reaction set $R$ and stoichiometric subspace $S_t$. Suppose the network has rank $s$ and $r$ reactions with $p$ reversible reaction pairs. Let the reaction set for an arbitrary spanning subnetwork be $F$ and let $\{d^{(1)}, d^{(2)}, \ldots, d^{(r-p-s)}\}$ be a set of corresponding spanning-subnetwork vectors. Then the corresponding isothermal mass action differential equations for the given network have the capacity to admit an unstable steady state having a positive real eigenvalue with its eigenvector in the stoichiometric subspace $S_t$, if and only if there exist a nonzero vector $\gamma \in S_t$, a vector $\mu \in \mathbb{R}^N$ which is sign compatible with $\gamma$, and also numbers $\xi_1, \xi_2, \ldots, \xi_{r-p-s}$, $\alpha_1, \alpha_2, \ldots, \alpha_{r-p-s}$ satisfying the following two conditions:

(i) For every reversible reaction

$$y_i \rightarrow y_j \in R \text{ with } y_i \rightarrow y_j \in F,$$

$$\sum_{L=1}^{r-p-s} [\xi_L (y_j \cdot \mu) + \alpha_L d^{(L)}_{i-j}] \gamma_{i-j} > 0$$

and

$$\sum_{L=1}^{r-p-s} [\xi_L (y_j \cdot \mu) + \alpha_L d^{(L)}_{i-j}] \gamma_{i-j} = 0.$$

(ii) For every irreversible reaction $y_i \rightarrow y_j \in R$,

$$\sum_{L=1}^{r-p-s} \xi_L d^{(L)}_{i-j} > 0$$

and

$$\sum_{L=1}^{r-p-s} [\xi_L (y_j \cdot \mu) + \alpha_L d^{(L)}_{i-j}] \gamma_{i-j} = 0.$$

If a set of solutions to the above conditions exists, the vectors $\gamma$, $\mu$, and the numbers $\xi_i, \alpha_i, i = 1, \ldots, r - p - s$, are used to construct a set of positive rate constants $\{k_{i-j}, i \rightarrow j \in R\}$ and an unstable steady state $c^*$ having a positive real eigenvalue $\lambda > 0$ with its corresponding eigenvector $\gamma \in S_t$. They are:

$$c^* = [c^*_1, \ldots, c^*_N] = \frac{\gamma}{\mu} = \left[ \frac{\gamma_1}{\mu_1}, \ldots, \frac{\gamma_N}{\mu_N} \right],$$

where $c^*_L$ is any positive real number, if $\gamma_L = \mu_L = 0$.

$$k_{i-j} = \frac{\lambda}{\prod_{L=1}^N (c^*_L)^{\gamma_L}}, \quad \forall y_i \rightarrow y_j \in R,$$
where $\lambda$ is any positive real number. The variable $\kappa_{i,j}$ is evaluated as follows:

For every irreversible reaction $i \rightarrow j \in R$,

$$\kappa_{i,j} = \sum_{L=1}^{r-p-s} \xi_L d_{i-j}^{(L)}.$$  

(7c)

For every reversible reaction $i \rightleftharpoons j \in R$ with $i \rightarrow j \in F$ and $y_i \cdot \mu \neq y_j \cdot \mu$,

$$\kappa_{i,j} = \frac{\sum_{L=1}^{r-p-s} [\xi_L (y_i \cdot \mu) + \alpha_L d_{i-j}^{(L)} - y_{i-j}]}{(y_j - y_i) \cdot \mu},$$  

(7d)

$$\kappa_{j,i} = \frac{\sum_{L=1}^{r-p-s} [\xi_L (y_j \cdot \mu) + \alpha_L d_{i-j}^{(L)} - y_{i-j}]}{(y_j - y_i) \cdot \mu}.$$  

(7e)

For all reversible reactions $i \rightleftharpoons j \in R$ with $i \rightarrow j \in F$ and $y_i \cdot \mu = y_j \cdot \mu$,

$$\kappa_{i,j} > 0, \quad \kappa_{j,i} > 0,$$  

(7f)

$$\kappa_{i-j} - \kappa_{j-i} = \sum_{L=1}^{r-p-s} \xi_L d_{i-j}^{(L)}.$$  

(7g)

3.2. Applications

Example 1

Consider network (1). By the spanning-subnetwork vector in (4) and the positive real eigenvalue condition, a set of inequalities and equations is constructed as follows:

For a reversible reaction $0 \rightleftharpoons A_1$ with $0 \rightarrow A_1 \in F$,

$$[\xi_1 \cdot 0 + \alpha_1] \cdot 0 - \gamma_{0 \rightarrow A_1} \text{ and } [\xi_1 \cdot \mu + \alpha_1] \cdot 0 - \gamma_{0 \rightarrow A_1} \text{ are s. c. w. } \mu_1.$$  

(8a)

For an irreversible reaction $A_1 \rightarrow A_2$,

$$[\xi_1 \cdot 0 + \alpha_1] \cdot 0 - \gamma_{0 \rightarrow A_1} \text{ and } [\xi_1 \cdot \mu + \alpha_1] \cdot 0 - \gamma_{0 \rightarrow A_1} \text{ are s. c. w. } \mu_1.$$  

(8b)

For an irreversible reaction $A_1 \rightarrow A_2$,

$$[\xi_1 + \alpha_1] - \gamma_{A_1 \rightarrow A_2} = 0.$$  

(8c)

For an irreversible reaction $A_1 \rightarrow A_2$,

$$[\xi_1 (2\mu_1 + \mu_2) + \alpha_1] - \gamma_{A_1 \rightarrow A_2} = 0.$$  

(8d)

From (6), the eigenvector $\gamma \in S_r$ is represented by

$$\gamma = \gamma_{0 \rightarrow A_1} (A_1 - 0) + \gamma_{A_1 \rightarrow A_2} (A_2 - A_1)$$  

$$+ \gamma_{A_2 \rightarrow A_3} (A_3 - A_2) = [A_1] \gamma_{0 \rightarrow A_1} - \gamma_{A_1 \rightarrow A_2} + \gamma_{A_2 \rightarrow A_3} A_1$$  

$$+ [A_2] (\gamma_{A_1 \rightarrow A_2} - \gamma_{A_2 \rightarrow A_3} A_2).$$

The condition requires that $\mu$ is $\gamma$. This implies that

$$\mu_1 \text{ is s. c. w. } \gamma_{0 \rightarrow A_1} - \gamma_{A_1 \rightarrow A_2} + \gamma_{A_2 \rightarrow A_3} A_1, \quad (9a)$$

$$\mu_2 \text{ is s. c. w. } \gamma_{A_1 \rightarrow A_2} - \gamma_{A_2 \rightarrow A_3} A_2.$$  

(9b)

The inequalities and equations (8) and (9) are solved to determine an unstable steady state with a real positive eigenvalue. From (8a), (9a) and (9b), the sign patterns of $\mu_1$ and $\mu_2$ are required to solve (8) and (9). Thus, it is divided into many cases by assuming the sign patterns, such as $\mu_1 > 0, \mu_2 > 0, \mu_1 > 0, \mu_2 = 0, \mu_1 > 0, \mu_2 < 0$. For each case it is not difficult to see that the system of equations (8) and (9) with the assumed sign pattern is linear. Indeed, this is always true for any reaction network with $r - p - s = 1$. Mathematically, a linear system of inequalities and equations can be solved by the Simplex method even when containing many unknowns. By this method we find a set of solutions to (8) and (9). It is

$$\mu_1 = -2, \quad \mu_2 = 1.5,$$  

$$\gamma_{0 \rightarrow A_1} = 1, \quad \gamma_{A_1 \rightarrow A_2} = 3, \quad \gamma_{A_2 \rightarrow A_3} = 0.$$  

(10)

Based on (7) and (10), we construct an unstable steady state $e^*$ having a positive real eigenvalue ($\lambda = 1$) with its eigenvector $\gamma \in S_r$ and a set of corresponding positive rate constants:

$$e^* = \frac{\gamma}{\mu} = \begin{bmatrix} \gamma_{0 \rightarrow A_1} \\ \mu_1 \\ \mu_2 \end{bmatrix} = \begin{bmatrix} 1 \\ \gamma_{A_1 \rightarrow A_2} \\ \gamma_{A_2 \rightarrow A_3} \end{bmatrix} = \begin{bmatrix} 1 - 3 + 0 \\ -2 \\ 1.5 \end{bmatrix} = \begin{bmatrix} 1, 2 \end{bmatrix}.$$  

(11a)

$$k_{0 \rightarrow A_1} = \frac{\lambda_{0 \rightarrow A_1}}{\sum_{L=1}^{2} (e_{1L}^2)\mu_1} = \frac{\lambda_{0 \rightarrow A_1}}{\mu_1} = \frac{\mu_1}{\mu_2} + \frac{\mu_1}{\mu_2} = 0.5.$$  

(11b)
Fig. 1. The phase portrait for the reaction network (1) with the rate constants given in (11).

\[ k_{A_1 \rightarrow 0} = \lambda \kappa_{A_1 \rightarrow 0} \prod_{l=1}^{2} (c_{l}^*)^{\gamma_{l}} = \frac{\lambda (1^{(-1/2)})}{(1^{1})(2)^{0}} = 0.5, \]  
\[ k_{A_1 \rightarrow A_2} = \lambda \kappa_{A_1 \rightarrow A_2} \prod_{l=1}^{2} (c_{l}^*)^{\gamma_{l}} = \frac{\lambda (\xi d_{A_1 \rightarrow A_2})}{c_{1}^{2}c_{0}^{2}} = \frac{1(6)(1)}{1^{1}(2)^{0}} = 6, \]  
\[ k_{A_2 \rightarrow A_3} = \lambda \kappa_{A_2 \rightarrow A_3} \prod_{l=1}^{2} (c_{l}^*)^{\gamma_{l}} = \frac{\lambda (\xi d_{A_2 \rightarrow A_3})}{c_{1}^{2}c_{1}^{2}} = \frac{1(6)(1)}{1^{2}(2)^{1}} = 3, \]  

The calculated steady state is one of the many states that are unstable.

The reaction network (1) has deficiency one. A deficiency one network can or can not admit multiple steady states. The deficiency one algorithm [13] is used to show that network (1) can not admit multiple steady states no matter what rate constants it might have. A perturbation of a steady state may grow, because of the instability, and approach eventually another attractor. It is interesting to know where an initial concentration, near the unstable steady state \( c^* \) in (11a) (K in Fig. 1) and the other four initial conditions (M, N, O and P) are surrounded by a clock-wise stable limit cycle (R → S → T → R → ...). The point L outside of the limit cycle also approaches it.

**Example 2**

Consider Higgins’ model [22] for glycolysis

\[ 0 \rightarrow A_1, \]
\[ A_1 + A_2 \rightarrow A_3 + A_4, \]
\[ A_3 \rightarrow A_2, \]
\[ A_2 \rightarrow A_4 + A_5, \]
\[ A_4 + A_6 \rightarrow A_7, \]
\[ A_7 \rightarrow A_6, \]  

and its corresponding mass action differential equations

\[ \frac{dc_1}{dt} = k_{0 \rightarrow A_1} - k_{A_1 \rightarrow A_2} c_1 c_2, \]
\[ \frac{dc_2}{dt} = -k_{A_1 \rightarrow A_2} c_1 c_2 + k_{A_3 \rightarrow A_2} c_3 - k_{A_2 \rightarrow A_4} c_2 + k_{A_5 \rightarrow A_2} c_5, \]
\[
\frac{dc_3}{dt} = k_{A_1 + A_2} - A_3 + A_4 c_3 c_2 - k_{A_5} - A_2 c_3,
\]
\[
\frac{dc_4}{dt} = k_{A_1 + A_2} - A_3 + A_4 c_1 c_2 + k_{A_2} - A_4 c_2 + k_{A_4} - A_5 c_4 c_5 - k_{A_4} - A_6 - A_4 c_4 c_6,
\]
\[
\frac{dc_5}{dt} = k_{A_2} - A_4 c_2 + k_{A_4} - A_5 c_4 c_5,
\]
\[
\frac{dc_6}{dt} = -k_{A_4} - A_6 - A_7 c_4 c_6 + k_{A_7} - A_6 c_7,
\]
\[
\frac{dc_7}{dt} = k_{A_2 + A_7} - A_4 c_6 - k_{A_7} - A_6 c_7. \tag{12b}
\]

For (12b) two mass conservation conditions are found:
\[
\frac{d(c_2 + c_3 + c_5)}{dt} = 0 \quad \text{and} \quad \frac{d(c_6 + c_7)}{dt} = 0. \tag{12c}
\]

Network (12a) has \( N = 7, r = 7, p = 1, s = 5 \). By choosing a spanning subnetwork, a corresponding spanning-subnetwork vector \( \mathbf{d} \) (with \( r = p = s = 5 \)) derived from (3) consists of the following elements:
\[
d_{0} \rightarrow A_1 = 1, \quad d_{A_1} \rightarrow A_2 + A_3 + A_4 = 1, \quad d_{A_1} \rightarrow A_5 = 1, \quad d_{A_2} \rightarrow A_4 + A_5 = 0, \quad d_{A_4} \rightarrow A_7 = 1, \quad d_{A_7} \rightarrow A_6 = 1. \tag{13a}
\]

By the above \( \mathbf{d} \) and the positive real eigenvalue conditions, a set of inequalities and equations is constructed:

For the reversible reaction \( A_2 \rightarrow A_4 + A_5 \) with \( A_2 \rightarrow A_4 + A_5 \in F \), \( [\xi_1 \cdot (\mu_3 + \alpha_1)] \cdot 0 = \gamma_{A_2} - A_4 + A_5 + \gamma_{A_2} - A_4 + A_5 \) is s. c. w. \( \mu_3 + \mu_5 - \mu_2 \).

For the irreversible reaction \( 0 \rightarrow A_1 \),
\[
\xi_1 > 0, \quad [\xi_1 \cdot (\mu + \alpha_1)] - \gamma_{0} - A_1 = 0.
\]

For the irreversible reaction \( A_1 + A_2 \rightarrow A_3 + A_4 \),
\[
\xi_1 > 0, \quad [\xi_1 (\mu_1 + \mu_2) + \alpha_1] - \gamma_{A_1 + A_2} - A_1 + A_4 = 0.
\]

For the irreversible reaction \( A_3 \rightarrow A_2 \),
\[
\xi_1 > 0, \quad [\xi_1 (\mu_3 + \alpha_1)] - \gamma_{A_1} - A_2 = 0.
\]

For the irreversible reaction \( A_4 + A_6 \rightarrow A_7 \),
\[
\xi_1 > 0, \quad [\xi_1 (\mu_4 + \mu_6) + \alpha_1] - \gamma_{A_4 + A_6} - A_7 = 0.
\]

For the irreversible reaction \( A_7 \rightarrow A_6 \),
\[
\xi_1 > 0, \quad [\xi_1 (\mu_7 + \alpha_1)] - \gamma_{A_7} - A_6 = 0. \tag{13b}
\]

From (6), an eigenvector \( \gamma \in S \) for network (12) is represented by
\[
\gamma = [A_1] (\gamma_{0} - A_1 - \gamma_{A_1} - A_2 - A_3 + A_4)
\]
\[
+ [A_2] (-\gamma_{A_1} - A_2 - A_3 + A_4 + \gamma_{A_2} - A_2 - A_4 + A_3)
\]
\[
+ [A_3] (\gamma_{A_1} + A_2 - A_4 - A_5 - \gamma_{A_3} - A_2)
\]
\[
+ [A_4] (\gamma_{A_1} + A_2 - A_4 + A_3 + \gamma_{A_2} - A_4 + A_5)
\]
\[
+ [A_5] (\gamma_{A_2} - A_4 + A_3)
\]
\[
+ [A_6] (-\gamma_{A_4} - A_6 - A_7 + \gamma_{A_2} - A_6)
\]
\[
+ [A_7] (\gamma_{A_4} + A_6 - A_7 - \gamma_{A_7} - A_6). \tag{14a}
\]

The \( \mathbf{P} \) is required to be s. c. w. \( \gamma \). This implies that
\[
\mu_1 = \text{s. c. w.} \quad \gamma_{0} - A_1 - \gamma_{A_1} - A_2 - A_3 + A_4,
\]
\[
\mu_2 = \text{s. c. w.} \quad -\gamma_{A_1} - A_2 - A_3 + A_4 + \gamma_{A_2} - A_2 - A_4 + A_3,
\]
\[
\mu_3 = \text{s. c. w.} \quad \gamma_{A_1} + A_2 - A_4 + A_3 - \gamma_{A_3} - A_2,
\]
\[
\mu_4 = \text{s. c. w.} \quad \gamma_{A_1} + A_2 - A_3 + A_4 + \gamma_{A_2} - A_4 + A_5
\]
\[
- \gamma_{A_4} - A_5 - A_7,
\]
\[
\mu_5 = \text{s. c. w.} \quad \gamma_{A_2} - A_4 + A_3,
\]
\[
\mu_6 = \text{s. c. w.} \quad -\gamma_{A_4} - A_6 - A_7 + \gamma_{A_2} - A_6,
\]
\[
\mu_7 = \text{s. c. w.} \quad \gamma_{A_4} + A_6 - A_7 - \gamma_{A_7} - A_6.
\]

By assuming the sign pattern of the vector \( \mu \), we find a set of solutions to the linear system of (13) and (14) by the Simplex method. It is
\[
\mu = [\mu_1, \ldots, \mu_7] = [-1, 6, 4, 8, -1, -6, 1],
\]
\[
\xi_1 = 1, \quad \alpha_1 = 0,
\]
\[
\gamma_{0} - A_1 = 0, \quad \gamma_{A_1 + A_2} - A_3 + A_4 = 5, \tag{15}
\]
\[
\gamma_{A_3} - A_2 = 4, \quad \gamma_{A_2 - A_4 + A_5} = -2,
\]
\[
\gamma_{A_4 + A_6 - A_7} = 2, \quad \gamma_{A_7 - A_6} = 1.
\]

Based on (7) and (15), we construct an unstable steady state \( c^* \) having a positive real eigenvalue \( \lambda = 1 \) with its eigenvector \( \gamma \in S \) and a set of corresponding positive rate constants. The calculated steady state
\[
0 \rightarrow A_1,
\]
\[
A_1 + A_2 \xrightarrow{1.2} A_3 + A_4,
\]
\[
A_3 \xrightarrow{4} A_2,
\]
\[
A_4 \xrightarrow{4} A_2,
\]
\[
A_6 \xrightarrow{3} A_7,
\]
\[
A_7 \xrightarrow{3} A_6.
\]
Fig. 2. The phase portrait for the reaction network (16a).

Fig. 3. The concentration-time plot for the reaction network (16a) with different rate constants $k_{A_1+A_6\to A_7}$.
Unstable Steady States in Chemical Reaction Networks

Fig. 4. The bifurcation plot ($k_{A_4 + A_6 \rightarrow A_7}$ vs. $c_6$) for the reaction network (16a).

$$A_2 \xrightarrow{12/8} A_4 + A_5,$$

$$A_4 + A_6 \xrightarrow{48} A_7,$$

$$A_7 \xrightarrow{1} A_6,$$

(16a)

$$c^* = [c_1^*, c_2^*, c_3^*, c_4^*, c_5^*, c_6^*, c_7^*]$$

$$= [5, 1/6, 0.25, 0.125, 2, 1/6, 1]$$

(16b)

is one of the many that are unstable.

Similar to example 1, the reaction network (12a) has deficiency one and can not admit multiple steady states no matter what rate constants it might have. Where does an initial concentration, near the unstable steady state $c^*$ in (16b) and satisfying the mass conservation conditions in (12c), go without approaching any other steady state? For network (16), four different initial conditions, satisfying the mass conservation conditions in (12c), are numerically simulated and the phase portrait $c_1$ vs. $c_6$ is shown in Figure 2. The unstable steady state $c^*$ in (16b) (I. C. 4) and the other two initial conditions (I. C. 2 and I. C. 3) are surrounded by a clockwise stable limit cycle ($R \rightarrow S \rightarrow T \rightarrow R \rightarrow \ldots$). The I. C. 1 outside of the limit cycle also approaches it.

Figure 3 shows the concentration ($c_6$)-time ($t$) plot with variation of the rate constant $k_{A_4 + A_6 \rightarrow A_7}$. When $k_{A_4 + A_6 \rightarrow A_7}$ equals to 12.9, the system admits a stable steady state; it gives rise to a stable limit cycle for $k_{A_4 + A_6 \rightarrow A_7}$ equal to 13.5, 25 and 48 with an increase of the oscillating cycle time and the amplitude of $c_6$ when increasing the rate constant $k_{A_4 + A_6 \rightarrow A_7}$. Figure 4 displays the bifurcation plot of $k_{A_4 + A_6 \rightarrow A_7}$ vs. $c_6$.

If $k_{A_4 + A_6 \rightarrow A_7}$ is smaller than 13, the system admits a stable steady state, and the stable state concentration $c_6$ almost remains unchanged. When $k_{A_4 + A_6 \rightarrow A_7}$ is greater than 13, the system exhibits a stable limit cycle. For $k_{A_4 + A_6 \rightarrow A_7}$ greater than about 20, the amplitude of $c_6$ in the limit cycle (indicated by the max. and min. value in Fig. 4) also remains almost unchanged. When $13 < k_{A_4 + A_6 \rightarrow A_7} < 20$, the amplitude of $c_6$ in the limit cycle increases with an increase of the rate constant $k_{A_4 + A_6 \rightarrow A_7}$.

For both examples 1 and 2, a perturbation of an unstable steady state approaches to a stable limit cycle. A steady state with a pair of complex conjugate eigenvalues would indicate a limit cycle appearing via a Hopf bifurcation. It would be nicer than the condition proposed in this work to have a condition for mass action kinetics having a steady state with a pair of complex conjugate eigenvalues. The existence of a limit cycle might be predicted directly by such a condition without a computer simulation.

Example 3

Consider the following Langmuir-Hinshelwood mechanism for a bimolecular reaction in an ideal
isothermal CSTR, in which the interphase resistance is neglected [23]:

\[ A_1 \rightleftharpoons 0 \rightleftharpoons A_2, \]

\[ A_6 \]

\[ A_1 + A_3 \rightleftharpoons A_4, \]

\[ A_2 + 2A_3 \rightleftharpoons 2A_5, \]

\[ A_4 + A_5 \rightleftharpoons A_6 + 2A_3. \]

(17a)

Species \( A_1 \) and \( A_2 \) are reactants; \( A_3 \) is a vacant active site; \( A_4 \) and \( A_5 \) are active sites adsorbed by \( A_1 \) and \( A_2 \), respectively; and \( A_6 \) is a product. The corresponding mass action differential equations for network (17a) are:

\[
\frac{dc_1}{dt} = -k_{A_1-}c_1 + k_{0->A_1} - k_{A_1+}c_1c_3
\]

\[ + k_{A_1-}c_2c_4, \]

\[
\frac{dc_2}{dt} = -k_{A_2-}c_2 + k_{0->A_2} - k_{A_2+}c_2c_3
\]

\[ + k_{A_2-}c_2c_5, \]

\[
\frac{dc_3}{dt} = -k_{A_1+}c_3 + k_{A_1-}c_1c_3
\]

\[ -2k_{A_2+}c_3c_2 + 2k_{A_3-}c_3c_2
\]

\[ + 2k_{A_4+}c_3c_4c_5, \]

\[
\frac{dc_4}{dt} = k_{A_1-}c_1c_3
\]

\[ - k_{A_4-}c_4 - k_{A_4+}c_4c_5, \]

\[
\frac{dc_5}{dt} = 2k_{A_1+}c_3c_2c_5
\]

\[ - 2k_{A_3-}c_3c_2c_5 - k_{A_5-}c_5c_4c_5, \]

\[
\frac{dc_6}{dt} = -k_{A_6-}c_6 + k_{A_5+}c_6 + 2k_{A_4+}c_6c_5. \]

(17b)

For (17b) a mass conservation condition is found:

\[
\frac{d(c_3 + c_4 + c_5)}{dt} = 0. \]

(17c)

Network (17a) has \( N = 6 \), \( r = 10 \), \( p = 4 \) and \( s = 5 \).

By choosing a spanning subnetwork, a corresponding spanning-subnetwork vector \( \mathbf{d} \) (\( r - p - s = 10 - 4 - 5 = 1 \)) is derived from (3). Its elements are:

\[
d_{A_1-} = 2, \quad d_{A_2-} = 1, \quad d_{A_3-} = 2,
\]

\[
d_{A_1+} = 2, \quad d_{A_3+} = 2, \quad d_{A_4+} = 2, \quad d_{A_5+} = 1, \quad d_{A_6+} = 2. \]

(18a)

By the above \( \mathbf{d} \) and the positive real eigenvalue condition, a set of inequalities and equations is constructed:

\[
\alpha_1(2) - \gamma_0 - A_1, \quad \alpha_2(1) - \gamma_0 - A_2
\]

\[ \xi_1(\mu_1 + \alpha_1)(2) - \gamma_0 - A_1 \] are s. c. w. \( \mu_1 \),

\[ \alpha_1(1) - \gamma_0 - A_2 \] and

\[ \xi_1(\mu_2 + \alpha_1)(1) - \gamma_0 - A_2 \] are s. c. w. \( \mu_2 \),

\[ \xi_1(\mu_1 + \alpha_3)(2) - \gamma_1 - A_1 \] and

\[ \xi_1(\mu_4 + \alpha_3)(1) - \gamma_1 - A_1 \] are s. c. w. \( \mu_4 - \mu_1, \mu_5, \)

\[ \xi_1(\mu_2 + 2\mu_3 + \alpha_3)(1) - \gamma_2 + 2A_3 - 2A_5 \] and

\[ \xi_1(2\mu_5 + \alpha_3)(1) - \gamma_2 + 2A_3 - 2A_5 \]

are s. c. w. \( \mu_5 - \mu_2 - 2\mu_3, \)

\[ \xi_1(2 \mu_4 + \alpha_3)(1) - \gamma_4 + A_5 - A_6 + 2A_4 = 0. \]

(18b)

According to (6), an eigenvector \( \gamma \in S_1 \) can be represented by:

\[ \gamma = [A_1][\gamma_0 - A_1 - \gamma_1 - A_1 - A_4] + [A_2][\gamma_0 - A_2 - \gamma_2 + 2A_3 - 2A_5] + [A_3][-\gamma_1 - A_1 - A_4 - 2\gamma_2 + 2A_3 - 2A_5 + 2\gamma_4 + A_5 - A_6 + A_3] + [A_4][\gamma_1 - A_1 - A_4 - A_6 + 2A_5] + [A_5][2\gamma_2 + 2A_3 - 2A_5 - \gamma_4 + A_5 - 2A_4] + [A_6][-\gamma_6 - 0 + \gamma_4 + A_5 - A_6 + 2A_4]. \]

(19a)

It is required that \( \mu \) is s. c. w. \( \gamma \). This implies that

\[ \mu_1 \text{ is s. c. w. } \gamma_0 - A_1 - \gamma_1 - A_4, \]

\[ \mu_2 \text{ is s. c. w. } \gamma_0 - A_2 - \gamma_2 + 2A_3 - 2A_5, \]

\[ \mu_3 \text{ is s. c. w. } -\gamma_1 - A_1 - A_4 - 2\gamma_2 + 2A_3 - 2A_5 + 2\gamma_4 + A_5 - A_6 + 2A_3, \]

\[ \mu_4 \text{ is s. c. w. } \gamma_1 - A_1 - A_4 - A_6 + 2A_5, \]

\[ \mu_5 \text{ is s. c. w. } 2\gamma_2 + 2A_3 - 2A_5 - \gamma_4 + A_5 - A_6 + 2A_3, \]

\[ \mu_6 \text{ is s. c. w. } -\gamma_6 - 0 + \gamma_4 + A_5 - A_6 + 2A_4. \]

(19b)

We find a set of solutions to (18b) and (19b). It is:

\[ \mu_1 = 0.00175, \quad \mu_2 = 0.001, \quad \mu_3 = -0.00225, \]

\[ \mu_4 = 0.001, \quad \mu_5 = -0.003, \quad \mu_6 = -0.0015, \]

\[ \xi_1 = 1, \quad \alpha_1 = 0.001, \]

\[ \gamma_0 - A_1 = 0.0005, \quad \gamma_0 - A_2 = -0.000875. \]
\[ \gamma_{A_1+A_3\rightarrow A_4} = 0, \quad \gamma_{A_2+2A_1\rightarrow 2A_3} = -0.0015, \]
\[ \gamma_{A_4+2A_5\rightarrow 2A_3} = -0.002, \quad \gamma_{A_6} = -0.001. \] 

Based on (7) and (20), we construct an unstable steady state \( c^* \) having a positive real eigenvalue \( (\lambda = 1) \) with its eigenvector \( \gamma \in S \) and a set of corresponding positive rate constants. The calculated steady state
\[
\begin{align*}
A_1 &\xrightarrow{\frac{3}{2.85714}} 0 \xrightarrow{\frac{2.875}{3}} A_2, \\
A_1 + A_3 &\xrightarrow{\frac{21}{0.33333}} A_4, \\
A_2 + 2A_3 &\xrightarrow{\frac{11.34}{3.6}} 2A_5, \\
A_4 + A_5 &\xrightarrow{\frac{3}{5}} A_6 + 2A_3,
\end{align*}
\]

(21a)
is one of the many that are unstable.

The reaction network (17a) has deficiency one, and the deficiency one algorithm shows that it has capacity to admit multiple steady states. The perturbation of the unstable steady state in (21b) grows and approaches two other stable steady states. In Fig. 5, the steady states and bistability occurring in network (21a) are illustrated as hysteresis with variation of \( k_0\rightarrow A_1 \). In reaction network terms, the rate constant \( k_0\rightarrow A_1 \) equals to the flow rate of the reactor multiplying the feed concentration of species \( A_1 \). Increasing the feed concentration of species \( A_1 \) increases the value of \( k_0\rightarrow A_1 \), and vice versa. The steady states \( c' \) (the lower points) and \( c'' \) (the upper points) are both stable and an unstable steady state \( c^* \) lies somewhere between \( c' \) and \( c'' \). The higher steady state concentration \( c_6 \) establishes for a lower \( k_0\rightarrow A_1 \) \((< 2.857)\); while for \( k_0\rightarrow A_1 \) higher than 3.032 the steady state associating with a lower concentration \( c_6 \) is obtained. When \( k_0\rightarrow A_1 \) is in between, a hysteresis loop containing three steady states, two stable ones and an unstable one, occurs and the steady state depends on the initial concentrations. This shows the nonlinear phenomenon that a higher input rate of reactant \( A_1 \) \((k_0\rightarrow A_1)\) generates a lower product \( A_6 \) output.

Figure 6 shows a two-parameter \((k_{A_1+A_3\rightarrow A_4}, k_0\rightarrow A_1)\) plane for different values of the rate constant \( k_{A_4+A_5\rightarrow A_6+2A_3} (= 3 \text{ and } 1)\) for network (21a). Inside the cusp region, there are three steady states, two stable ones and an unstable one. They display the inside regions of a hysteresis loop similar to Figure 5. Right on the curves of the cusp, there are two steady states, a stable one and a saddle-node bifurcation. They rep-
resent the two end points of a hysteresis loop. Only a single steady state exists outside the cusp region in Fig. 6, which displays the outside region of a hysteresis loop. Figure 6 shows that, to maintain the existence of the steady state multiplicity under a fixed rate constant \( k_{A_4 + A_5 \rightarrow A_6 + 2A_3} \), the larger the rate constant \( k_{A_1 + A_3 \rightarrow A_4} \) is, the smaller the rate constant \( k_{0 \rightarrow A_1} \) is required and the wider the range of it exists. To maintain the existence of the steady state multiplicity under a larger rate constant \( k_{A_4 + A_5 \rightarrow A_6 + 2A_3} \), it is required to increase both the rate constant \( k_{0 \rightarrow A_1} \) and \( k_{A_1 + A_3 \rightarrow A_4} \).

4. Conclusions

The positive real eigenvalue condition provides a necessary and sufficient condition for the determination of an unstable steady state having a positive real eigenvalue for mass action reaction networks of any deficiency. The formula given in (7) can be used to construct a set of positive rate constants and an unstable steady state. Three deficiency one reaction networks are determined to have an unstable steady state with a positive real eigenvalue. Two exhibit sustained oscillations by approaching a stable limit cycle, and the other one admits multiple steady states.

The condition proposed in this work can be applied to a reaction network of any deficiency, it is particularly useful for a deficiency one network. The systems of inequalities and equations derived for a deficiency one network are linear and they can be solved by the Simplex method. The examples studied in this paper belong to such a category. When a chemical or a biological system involving many species with complex reactions is considered, this proposed method shows its advantages.

For networks having a deficiency higher than one, the derived system of inequalities and equations might become nonlinear and might not be easily solved. For them the strategy used in the subnetwork analysis [24] might be helpful. The idea is to study their deficiency one subnetworks by the positive real eigenvalue condition. If a deficiency one subnetwork is determined to admit an unstable steady state with a positive real eigenvalue, then the unstable steady state might also generate instability for the original network and even for a family of reaction networks.